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Perceived Stress and Surgical Wound Cytokine Patterns

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Perceived Stress and Surgical Wound Cytokine Patterns

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy at Virginia Commonwealth University

By

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In loving memory of my mother, Barbara Valentina Sage, who courageously overcame so many hardships and stresses, yet taught me the value of hard work and perseverance. Her amazing spirit was with me to the very end.

And Sophie Lee Lucas, who inspires me more than she knows and who constantly reminds me that anything is possible if you only believe. I love you!!

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Abstract:

PERCEIVED STRESS AND SURGICAL WOUND CYTOKINE PATTERNS

By Valentina Sage Lucas, RN, MS, ANP-BC

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2012

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Normal wound healing is a complex process that occurs in overlapping phases and depends upon interactions of the patient, environment and a large number of cells, growth factors, cytokines, chemokines, and other biochemical mediators. Psychological stress has been shown to adversely affect the normal wound healing process through its impact on cellular immunity. Cellular immunity impacts wound healing through the production and regulation of many of the above biochemical mediators of wound healing. The purpose of this pilot study was to examine the relationships among pre- and post-operative psychological stress experienced by women who were undergoing either immediate or delayed breast reconstruction following mastectomy for breast cancer and influence of that stress on wound healing, specifically the biochemical mediators of wound healing in the local wound environment. An integration of Lazarus and Folkman's cognitive appraisal model of stress and coping and the psychoneuroimmunology model

proposed by McCain, Gray, Walter and Robins (2005) served as the theoretical framework for the research. A descriptive non-experimental design was used, with samples collected over time to describe biochemical patterns in surgical wounds of women undergoing autologous breast reconstruction. Biochemical data were collected preoperatively, as well as at 24, 48, 72 and 96 hours postoperatively. Psychological stress instruments were administered pre-operatively and 48 hours post-operatively. Although subjects overall displayed low levels of psychological stress, meaningful wound fluid biochemical mediator patterns were detected. This study adds to our knowledge concerning wound fluid chemical mediators present in the local wound environment over time.

CHAPTER 1

Introduction

Research shows a strong relationship between psychological stress and health, including but not limited to delayed wound healing (Broadbent, Petrie, Alley & Booth, 2003; Glaser, Kieclt-Glaser, Marchucha, MacCallum, Laskowski & Malarkey, 1999; Ebrecht, Hestall, Kirtley, Taylor, Dyson & Weinman, 2003), poor surgical outcomes (Linn, Linn & Klimas, 1988), and decreasing immune system functioning (Padgett & Glaser, 2003; Lusk & Lash, 2005; Starkweather, 2007; Von Ah, Kaan & Carpenter, 2007). Stress has been implicated in the lay literature as the cause of vague body aches, headaches, stomach upset, loss of interest in sex, depressed mood, and eating disorders. Most diseases are caused by multiple factors, so it is most likely that stress alone does not cause poor health outcomes, but is one factor that adversely affects health.

The diagnosis of cancer can produce an “existential plight”, bringing feelings of shock, disbelief, and emotional turmoil (Weisman & Worden, 1976). Surgical procedures have been identified as a “high-stakes” stressor (Borah, Rankin & Wey, 1999). Women living with a diagnosis of breast cancer and facing mastectomy and subsequent surgical reconstruction may experience psychological reactions produced by the anticipation of surgery and treatment for their disease. As patients navigate numerous medical “stops” on their way to surgery, such as radiology studies, biopsy, multiple physical exams by a variety of consultants and laboratory studies (Frierson & Anderson, 2006), they are

dealing with thoughts of body image, family issues, career and employment concerns, and thoughts of mortality, all of which can cause significant stress and anxiety. One study reported as much as 50% of women with breast cancer experience sub-clinical symptoms of Post Traumatic Stress Disorder (Levine, Eckhardt & Targ, 2005).

Certainly women with breast cancer experience stress. Extended activation of the stress response can lead to chronic activation of the hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullary axis, leading to chronic production of glucocorticoid hormones and catecholamines, resulting in a dysregulation of immune system functioning (Padgett & Glaser, 2003). Cellular immunity has an important role in the regulation of wound healing through the production and regulation of cytokines, and chemokines, which control and regulate many processes in the body, of which wound healing is one. This dissertation will first present in Chapter 2 a review of the literature as it pertains to psychological stress and wound healing in humans; this chapter was published in *Wounds* in 2011. A basic review of the phases of wound healing is included in this publication. Chapter 3 is the defended and approved IRB research proposal, outlining the research plan, design, and steps taken to insure protection of research subjects for the dissertation research. The findings of this research are detailed in the manuscript presented as Chapter 4. This work is a step forward to understanding relationships of various chemical mediators in the local wound environment and to our knowledge, is the first to characterized surgical wound fluid cytokine patterns over time.

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Psychological Stress and Wound Healing in Humans: What We Know

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Abstract: The phenomenon of stress is a common human experience frequently blamed for much of the ill health individuals experience. Much focus has been given to the effect of stress on health and wellness. Research demonstrates a strong relationship between psychological stress and health including, but not limited to, poor surgical outcomes and a decrease in immune system functioning. The skin is the largest organ of the human body and is responsible for thermoregulation, vitamin D production, and protection from fluid loss, pathogens, ultraviolet radiation, and mechanical injury. The skin contains a vast supply of sensory nerves, providing sensory input on pain, temperature, pressure, and pleasure. Timely wound healing is of utmost importance because of the skin's vital protective and regulatory functions. Psychological stress has been shown to negatively impact wound healing, both directly and indirectly. The purpose of this review is to identify existing knowledge about the relationship between psychological stress and wound healing in order to provide the best evidence currently available on which to base recommendations for future research and to guide practice.

The phenomenon of stress is a common human experience frequently blamed for much of the ill health individuals experience. Much focus has been given to the effect of stress on health and wellness. Stress can be physical or psychological, acute or chronic. Stress is often thought of as the human response to a stimulus or more specifically a “stressor” in one’s environment. The response is often based on an individual’s appraisal of the situation, the individual’s coping behaviors, and the resources available to the individual. Lazarus and Folkman¹ specifically define stress as a “relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her wellbeing.” Appraisal and coping are key to this definition and lead to the subjective experience of stress. The degree of perceived threat determines the magnitude of the stress response to the environmental event. When individuals can no longer cope with stressful situations, affective, behavioral, and

physiological changes result.² Research demonstrates a strong relationship between psychological stress and health including, but not limited to, alteration in immune system functioning,³⁻⁶ poor surgical outcomes,⁷ alterations in metabolism, increased risk of obesity,^{8,9} and increased risk of developing cardiovascular disease.^{10,11}

The skin is the largest organ of the human body and is responsible for protection from pathogens, ultraviolet radiation, and mechanical injury. Additionally, the skin provides protection from fluid loss and has an active role in thermoregulation, as well as vitamin D production. The slightly acidic pH of the skin serves as a protective barrier against bacterial and fungal invasions. The skin contains a vast supply of sensory nerves that provide sensory input regarding pain, temperature, pressure, and pleasure.^{12,13} Timely wound healing is of utmost importance because of the skin's vital protective and regulatory functions.

Wounds of the skin typically progress in a predictable and timely manner. When the normal phases of wound healing are interrupted, chronic wounds develop, which leads to an increased risk of infection, prolonged hospital stays, and decreased quality of life. Chronic wounds account for a significant amount of healthcare spending in the United States, amounting to an estimated \$5 to \$9 billion each year.¹⁴

Stress has been shown to have a negative impact on wound healing.¹⁵⁻¹⁷ Although both the direct and indirect mechanisms of stress may be responsible for slowed healing, the most prominent impact is through the effects of stress on cellular immunity. Cellular immunity has an important role in the regulation of wound healing through the production and regulation of pro-inflammatory and anti-inflammatory cytokines. Cytokines, specifically platelet derived growth factor (PDGF), tumor necrosis factor (TNF- α), interferon-gamma (IFN- γ), various interleukins (IL-1 α , IL-1 β , IL-6, IL-8), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and transforming growth factor beta (TGF- β) potentially mediate many of the complex interactions involved in wound healing.

The purpose of this review is to identify existing knowledge about the relationship between psychological stress and wound healing in order to provide the best evidence currently available on which to base recommendations for future research and to guide practice.

How Wounds Heal

A wound can be defined "a disruption of the integrity and function of tissues in the body"¹⁸ and can be further

described according to its etiology (surgical, venous, neuropathic etc.), location (lower extremity, abdominal, foot, etc.) or by the duration (acute versus chronic).¹⁹ Normal cutaneous wound healing is a complex process that occurs in overlapping phases and depends upon interactions not only between the person and environment, but on multiple interactions among a large number of cells and chemical mediators including cytokines, hormones, and neurotransmitters. These phases do not occur in isolation, but are dynamic and overlapping. Even so, wound healing can be characterized by three phases: inflammatory, proliferative, and remodeling. The inflammatory phase begins within seconds of injury and lasts anywhere from 2-5 days. Blood vessel disruption activates platelets and triggers the release of clotting factors. The occurrence of vasoconstriction and platelet aggregation stops bleeding and provides a provisional matrix for cellular migration into the injured area.²⁰⁻²² The large number of platelets in the clot will degranulate and release numerous growth factors and cytokines.

Approximately 24 hours after injury, neutrophils and macrophages begin to remove nonviable tissue, debris, and bacteria from the wound through the release of enzymes and phagocytosis.²³ In addition to cleaning up the wound bed of nonviable tissue, macrophages and neutrophils have been shown to express several proinflammatory cytokines. Proinflammatory cytokines are some of the earliest signals to activate and recruit inflammatory cells and fibroblasts to the injury site causing inflammation and vasodilatation, which increases blood vessel permeability and allows easy passage of fluid and phagocytes. Platelet derived growth factor (PDGF) is released by the platelets, stimulating the growth of blood vessels and new structural tissue.^{20,24,25} Cytokines are also responsible for activation of keratinocytes found at wound edges, hair follicles, sebaceous and sweat glands. Keratinocytes are the most prevalent cell type of epithelium and begin to migrate and proliferate within 24 hours after injury, paving the way for the formation of new epithelium.²⁶⁻²⁸ This process continues on into the proliferative phase of wound healing.

Over the next 2 days to 3 weeks, the proliferative phase begins. Fibroblasts and other cell types begin to lay down the ground substances and collagen fibers in the site of injury. Various chemical mediators such as PDGF stimulate angiogenesis, which is marked by the formation of granulation tissue consisting of new capillary loops in a matrix of collagen and ground substance. Keratinocytes are actively carrying out re-epithelializa-

tion. Wound contraction is accomplished by the work of the myofibroblasts. The wound is considered closed with the establishment of a new epidermal covering.²⁹ The basement membrane, between the epidermis and the dermis, is typically repaired 7–9 days after re-epithelialization and is essential for the restoration of skin integrity and function.²²

Over the next 3 weeks to 2 years, the final phase of wound healing takes place. In the maturation or remodeling phase, type III collagen is gradually replaced with type I. Tensile strength increases as the collagen fibers reorganize. Healed wounds result in a scar, which differs somewhat from the original tissue, and has approximately 70%–80% of its original tensile strength.²⁵

KEYPOINTS

- For inclusion in this review, all studies had to have at least one measure of stress, such as the Perceived Stress Scale (PSS). Studies exploring the relationship between other psychological factors, such as depression or anxiety, were not included in this review

The Stress Response

Often when we think of the stress response, we think of the “Fight or Flight” response, whereby stressful events trigger simultaneous activation of both the hypothalamic-pituitary-adrenal (HPA) axis in the central nervous system and the sympatho-adrenomedullary (SAM) axis in the sympathetic nervous system. Activation of the SAM axis stimulates the release of the catecholamines epinephrine and norepinephrine, leading to an increased heart rate, increased blood flow to skeletal muscles, and an elevation in glucose metabolism. The SAM pathway is faster and has a more immediate physiological effect. Activation of the SAM axis activates the inflammatory response.

Activation of the HPA axis activates the release of corticotrophin-releasing hormone (CRH) from the hypothalamus and CRH then stimulates the release of adrenocorticotropin (ACTH) from the anterior pituitary, which in turn triggers the release of the glucocorticoids from the adrenal glands. In humans, this glucocorticoid is cortisol. Normal levels of glucocorticoids are believed to be immunomodulatory. However, when stress increases levels of glucocorticoids, suppression of inflammatory and immune responses occur.³⁰ Cortisol has been shown to decrease circulating leukocytes and inhibit the migration of leukocytes to the site of injury or infection by

KEYPOINTS

- When individuals can no longer cope with stressful situations, affective, behavioral, and physiological changes result
- Although both the direct and indirect mechanisms of stress may be responsible for slowed healing, the most prominent impact is through the effects of stress on cellular immunity mediated by cytokines and growth factors

decreasing capillary permeability and inhibiting chemotaxis. Elevated cortisol levels have been found to inhibit production of T cell-derived cytokines, such as interleukin 1.^{4,31}

Stress and Wound Healing

It is well established that psychological stress modulates immune system functioning and a fully functioning immune system is integral to timely and effective wound healing. Numerous studies have explored the relationship between psychological stress and wound healing. Although both human and murine models have been utilized in studying stress and wound healing, only studies involving human subjects will be included here. For inclusion in this review, all studies had to have at least one measure of stress, such as the Perceived Stress Scale (PSS). Studies exploring the relationship between other psychological factors, such as depression or anxiety, were not included in this review.

Systemic factors known to have negative effect on wound healing were considered exclusion criteria and were similar across all studies. Exclusion criteria most commonly cited included tobacco use, diabetes, peripheral vascular disease (PVD), cardiovascular disease (CVD), difficulty with wound healing in the past, immunologically related problems, recent surgeries, previous psychiatric illnesses, and use of anti-inflammatory medications with obvious immunological effects. Smokers and individuals with frequent alcohol consumption were often excluded. Wound types consisted of experimentally created wounds (eg, punch biopsy, blister) or preexisting clinical wounds (surgical and leg ulcers). Research utilized a variety of measures to evaluate the relationship between psychological stresses and wound healing. This makes a comparison of studies somewhat difficult since outcome measures varied. For the purpose of this review, findings are grouped based on how healing was assessed.

Digital Photography

The most simple and straightforward method of wound assessment is the use of digital photography. In a study of the effectiveness of a 3-month exercise intervention program on wound healing, neuroendocrine function, and perceived life stress, Emery et al³² used digital photography to assess wound healing. The wound healing outcome measure was the ratio between the areas of a standardized black dot applied next to the wound. Photos were taken on a schedule and wound healing was documented and considered positive as the size of the wound decreased in relation to the applied dot. A sample of 28 healthy men and women were randomized to either an exercise intervention group or a control group. The wounds of individuals who participated in regular exercise healed significantly faster (mean = 29.2, SE 9.0 days). All wounds for both groups healed by week 7. Interestingly, this study found that exercise improved healing yet did very little to improve self-reported stress on the PSS. Salivary cortisol was elevated in the exercise group but did not change in the nonexercise group. The authors proposed that the increased responsiveness of cortisol to stress following exercise suggests that exercise contributes to enhanced neuroendocrine responsiveness.³²

High Resolution Ultrasound

Ultrasounds use sound waves to produce images of soft tissue anatomy. Basically, a probe is placed over the area of interest and transmits sound waves into the body. When these sound waves hit a boundary between acoustically different tissues, such as bone versus soft tissue, a proportion of the energy, known as an echo, is reflected back.³³ High-resolution ultrasound typically involves frequencies of 15 megahertz (MHz) or higher. Depth of penetration is lost with higher frequencies, but resolution is improved. High-resolution ultrasound is useful in that it can show the fluid content of various tissues. Greater fluid content results in a decrease in the echogenicity in the tissue being observed. The use of high-resolution ultrasound has been shown to be effective in assessing dermal wounds.³⁴⁻³⁶

Ebrecht et al¹⁷ used a prospective, longitudinal, observational design to examine the relationship between perceived life stress and impaired wound healing. Psychometric testing included the PSS, the General Health Questionnaire, and the UCLA Loneliness scale. Four 4-mm punch biopsy wounds were placed on the upper arm of nonsmoking males, and wound healing was

evaluated using high-resolution ultrasound. A significant negative correlation between speed of wound healing and both the PSS scores ($r = -0.59$; $P < 0.01$) and the General Health Questionnaire was found. Morning cortisol levels were highest in those individual whose wounds were the slowest to heal.

Weinman et al³⁷ utilized high-resolution ultrasound in evaluating the healing of wounds placed on the hard palate. A prospective, longitudinal design with random assignment was used to test the ability of a disclosure intervention to lower psychological stress associated with a traumatic experience and to improve healing. While the disclosure intervention did not have a significant effect on the PSS scores, participants who wrote about emotional experiences had significantly faster wound healing times (wounds ~11% smaller) than the control group.

Hydrogen Peroxide Foaming Test

Two studies identified for this review utilized the hydrogen peroxide response test to assess wound healing. This method measures the quality of the epithelial barrier. The process involves the application of a small amount of hydrogen peroxide to the wounded area. Catalase, an enzyme found in connective tissue, liberates oxygen gas and water from the hydrogen peroxide. If the epithelial layer is disrupted, then foaming is visible at the wound site as oxygen is liberated. If the epithelial layer is intact, the diffusion of hydrogen peroxide does not take place and therefore little or no foaming is visible.³⁸⁻⁴⁰ Therefore, if there is no foaming present after application, the wound is considered healed.

Marucha et al⁴⁰ evaluated the effects of examination stress on dental students' ability to heal a mucosal wound. Each subject served as his or her own control. Two wounds were placed on the hard palate, the first during summer vacation. The second wound was placed on the contralateral side 3 days before the first major examination of the term. Daily photographs and foaming response to hydrogen peroxide were used to measure healing. Students took significantly longer to heal during the examination period (mean 7.82 days) compared to the vacation period (mean 10.91 days) [$F(1,10) = 28.47$; $P < 0.001$]. Students scored higher on the PSS and had lower whole blood IL-1 β levels during examinations. The decrease in IL-1 β demonstrates the decrease in immune function and a possible mechanism underlying the relationship of examination stress to wound healing.

Kiecolt-Glaser et al³⁸ used hydrogen peroxide foam

test to evaluate the effects of psychological stress caused by caring for a relative with Alzheimer's disease on wound healing. Healthy female caregivers and controls were studied simultaneously, matched for age and income. Complete wound healing of a 3.5-mm punch biopsy, as indicated by lack of foaming after the application of hydrogen peroxide, took significantly longer in caregivers (mean 48.7 days, SE = 2.9) than controls (mean 39.3 days, SE = 3.0). Wound healing took on average 9 days longer in caregivers than in controls. In addition, caregivers reported significantly more stress on the PSS than did control participants on study entry (20.5, SE = 1.6 versus 13.7, SE = 1.5, $P < 0.002$). There was no significant change during the study, nor was there an interaction between group and time.

Wound Fluid Analysis

Since numerous chemical mediators and cell types are involved in the complex process of wound healing, analysis of wound fluid provides some insight into the extracellular environment of the wound.^{41,42} Wound fluid characterization provides the opportunity to obtain information reflecting the status of the wound at specific time points, and holds potential for the development of specific biomarkers of impaired healing.⁴³ Analysis of wound fluid provides an opportunity to potentially connect the mechanisms of psychological stress to cellular mechanisms in the local wound site.

In one prospective, longitudinal, observational study, Glaser et al¹⁶ assessed the relationship between perceived life stress and the production of proinflammatory cytokines at the wound site. Blister wounds were created on 36 healthy female subjects and wound fluid was analyzed. Psychological tests included the 10-item Perceived Stress Scale (PSS), the Positive and Negative Affect Schedule, and the Psychiatric Epidemiological Research Inventory Life Events Scale. Health-related behaviors were also assessed. Women reporting greater stress had lower production of IL-1 α [$F(1,32) = 5.73, P < 0.03$] and IL-8 [$F(1,32) = 5.31, P < 0.03$] in the wound blister fluid.

In a second prospective longitudinal study, Broadbent et al¹⁵ evaluated the relationship between preoperative stress and worry and wound healing in patients undergoing routine surgical repair for an inguinal hernia. Psychological measures included the PSS, the Worry Visual Analogue Scale, and the Mental Health Index. Wound healing was determined through changes in cytokine profiles of surgical wound fluid collected from

the routine manovac drain placed at the time of surgery. Higher reported preoperative psychological stress predicted impaired cellular wound repair processes in the early postoperative period. For example, higher preoperative stress significantly predicted lower levels of IL-1 in the wound fluid ($\beta = -0.44; P = 0.03$). Greater worry about the surgery predicted lower levels of matrix metalloproteinase-9 in the wound fluid ($\beta = -0.38; P = 0.03$), a more painful recovery ($\beta = 0.51; P = 0.002$), and slower recovery ($\beta = 0.43; P = 0.01$). Neither stress nor worry predicted lower levels of IL-6 levels in the wound fluid. Interestingly, researchers did not exclude smokers. Smokers had higher levels of peripheral blood MMP-9 concentrations. Prolonged MMP-9 elevation has been associated with chronic non-healing wounds.⁴⁴ Lower cytokines were also associated with higher cortisol levels.

Lastly, Kiecolt-Glaser et al⁴⁵ explored the effect of hostile marital relationships on wound healing. A group of 42 healthy married couples, aged 22 to 77 years (mean 37.04) who were married a mean of 12.55 years, were enrolled in an experimental cross-over study. Couples were engaged in a structured social support interaction during the first phase of the study, and in the second phase, were asked to discuss a marital disagreement. Psychological evaluations included the Positive and Negative Affect Schedule (PANAS) and the Marital Adjustment Test, which provided data on marital satisfaction (higher scores indicate higher satisfaction). The Rapid Marital Coding System provided data on behavior during both phases of the study. Wound fluid was evaluated for changes in cytokine levels. In addition, wound healing was measured by the rate of transepidermal water loss (TEWL) using an evaporimeter, which is a non-invasive objective method to evaluate changes in the stratum corneum barrier function of the skin.⁴⁶ They found that couples' blister wounds healed more slowly following a single 30-minute marital conflict discussion

KEYPOINTS

- This review provides but a glimpse of what is known and what is still left to be discovered in the exciting area of psychological stress and wound healing. Other psychological mediators, such as depression and anxiety, have been shown to slow wound healing and should be explored further
- Future work to examine the exact mechanisms of pro-inflammatory and anti-inflammatory cytokines will shed additional light on the mechanisms of stress on wound healing

in a controlled setting in comparison to healing rates following supportive interactions ($P = 0.01$). Wound fluid cytokines (ie, IL-6, TNF- α , and IL-1 β) were also lower after conflict in comparison to social support. Additionally, participants with high-hostility behavior healed more slowly than the low-hostility behavior group ($P = 0.03$).

Conclusion

Clearly, there are substantial data in human studies to suggest that psychological stress and the subsequent effect on immune system disruption can impact wound healing. The purpose of this article is to serve as a foundation and a review of the existing literature on the relationship of psychological stress and wound healing in humans. Additionally, a review of available tools to measure wound healing for nursing research was presented. This article provides but a glimpse of what is known and what is still left to be discovered in the exciting area of psychological stress and wound healing. Other psychological mediators, such as depression and anxiety, have been shown to slow wound healing⁴⁷⁻⁴⁹ and should be explored further. Psychological stress can lead to unhealthy behaviors which may impact wound healing such as smoking,⁵⁰ poor nutrition,⁵¹ and altered sleep.⁵² For example, Rose et al⁵² point out that stress can negatively impact sleep, leading to disturbed sleep patterns and a reduction in growth hormones, which may down-regulate the tissue repair response.

Although numerous factors play a role in whether or not a wound heals, such as nutrition, underlying health conditions and appropriate care, it is clear that cytokines play a crucial role as well. If dysregulation of the various cytokines occurs, a potential disruption of normal wound healing results, leading to delayed healing and increased risk of infection and wound complications. Future work to examine the exact mechanisms of pro-inflammatory and anti-inflammatory cytokines will shed additional light on the mechanisms of stress on wound healing. Examination of wound fluid holds the potential to identify biomarkers of wound healing and subsequently provide diagnostic information on the wound environment to improve treatment modalities.

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VCU RESEARCH PLAN TEMPLATE

Use of this template is required to provide your VCU Research Plan to the IRB. Your responses should be written in terms for the non-scientist to understand. If a detailed research protocol (e.g., sponsor's protocol) exists, you may reference that protocol. **NOTE: If that protocol does not address all of the issues outlined in each Section Heading, you must address the remaining issues in this Plan. It is NOT acceptable to reference a research funding proposal.**

ALL Sections of the Human Subjects Instructions must be completed with the exception of the Section entitled "Special Consent Provisions." Complete that Section if applicable. When other Sections are not applicable, list the Section Heading and indicate "N/A."

NOTE: The Research Plan is required with ALL submissions and MUST follow the template, and include version number or date, and page numbers.

DO NOT DELETE SECTION HEADINGS OR THE INSTRUCTIONS.

I. Title

A Pilot Study of Perceived Stress and Surgical Wound Cytokine Patterns

II. STAFFING

A. In the table below (add additional rows as needed), indicate: (1) key project personnel including the principal investigator and individuals from other institutions, (2) their qualifications, and (3) a brief description of their responsibilities.

NAME OF INDIVIDUAL	QUALIFICATIONS	RESPONSIBILITIES
Nancy McCain	DSN, RN, FAAN	Faculty Advisor, Principle Investigator
Valentina S. Lucas	MS, RN, ANP-BC, PhD candidate	Doctoral Candidate, Student Investigator
Andrea Pozez	MD	Co-investigator, Committee Member
R.K. Elswick	PhD, Associate Professor Biostatistics	Co-investigator, Committee Member
Cindy Munro	PhD, RN, ANP-BC, FAAN, Nursing Alumni Endowed Professor	Co-investigator, Committee Member

B. Describe the process that you will use to ensure that all persons assisting with the research are adequately informed about the protocol and their research-related duties and functions.

The student and the principal investigator will meet approximately monthly throughout the proposal and dissertation process to discuss study processes. Monthly communication between each investigator will ensure that they are adequately informed about the protocol and each investigator's study-related duties and functions.

III. CONFLICT OF INTEREST

Describe how the principal investigator and sub/co-investigators might benefit from the subject's participation in this project or completion of the project in general. Do not describe (1) academic recognition such as publications or (2) grant or contract based support of VCU salary commensurate with the professional effort required for the conduct of the project

The principal investigator and the student investigator for this pilot study will not benefit financially from subjects' participation in the study or from completion of this project

IV. RESOURCES

Briefly describe the resources committed to this project including: (1) time available to conduct and complete the research, (2) facilities where you will conduct the research, (3) availability of medical or psychological resources that participants might require as a consequence of the research (if applicable), and (4) financial support.

The student investigator is conducting this pilot study as her doctoral dissertation study and therefore is committed through dissertation hours as required by the university. Recruitment will occur through the VCU Health System Plastic Surgery Faculty Practice. Resources also include use of the Center for Biobehavioral Clinical Research (CBCR) (supported by Center of Excellence grant #P30 NR011403, 2009-2014; R. Pickler, PI) in Virginia Commonwealth University's School of Nursing. The purpose of the CBCR is to enhance the research programs related to improving biobehavioral outcomes at Virginia Commonwealth University (VCU) School of Nursing. Center research focuses on the advancement of biobehavioral clinical science through research related to biobehavioral mechanisms, management, and outcomes of complex constellations of symptoms, including the commonly occurring symptom of fatigue. The pilot study will be funded by the PIs School of Nursing research account.

V. HYPOTHESIS

Briefly state the problem, background, importance of the research, and goals of the proposed project.

Normal wound healing is a complex process that occurs in orderly and overlapping phases. However, when these phases of wound healing are interrupted, chronic wounds develop, leading to increased risk of infection, increased hospital stays, and decreased quality of life. Chronic wounds account for a significant amount of healthcare spending in the United States, accounting for an estimated \$5 to \$9 billion each year (Mandracchi, John, & Sanders, 2001).

Psychological stress has been shown to adversely affect the normal wound healing process through its impact on cellular immunity. Cellular immunity has an important role in the regulation of wound healing through the production and regulation of pro-inflammatory and anti-inflammatory cytokines. The diagnosis of breast cancer is identified as a major life stressor (Kiecolt-Glaser & Glaser, 1994). Some studies indicate up to 80% of these women report significant stress during their initial treatment (Irvine, Vincent, Graydon & Bubela, 1998). The goal of this pilot study project is to examine pre- and post-operative psychological stress experienced by women who are undergoing mastectomy with immediate reconstruction or who have undergone mastectomy and are having delayed reconstruction and the potential impact on wound healing, specifically the chemical mediators of wound healing in the local wound environment.

VI. SPECIFIC AIMS

The aim of the pilot study is to describe the relationships psychological stress and post-operative peripheral blood cytokines and wound fluid cytokines among women undergoing mastectomy and immediate reconstruction surgery for breast cancer.

VII. BACKGROUND AND SIGNIFICANCE

Include information regarding pre-clinical and early human studies. Attach appropriate citations.

Normal wound healing is a complex process that occurs in phases and depends upon interactions not only between the patient and environment, but on multiple interactions among a large number of cells and chemical mediators including cytokines, hormones and neurotransmitters. Acute wounds, such as traumatic injuries and surgically created wounds often progress through orderly stages of wound healing and heal in a timely fashion. Full thickness wound repair can easily be broken down into four phases, the hemostasis phase, the inflammatory phase, the proliferative phase and the remodeling phase. The phases are not simple linear events but overlap in time to some degree (Li, Chen, & Kirsner, 2007). The hemostasis phase, lasting from the moment of injury to approximately 3 hours, is characterized by vasoconstriction and an inflow of clotting factors and platelets, leading to platelet aggregation and formation of a hematoma, which stops bleeding and provides a provisional matrix for cellular migration into the wounded site (Mehendale & Martin, 2001). Platelets in the clot will degranulate and release numerous growth factors and cytokines. Platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF- β) are present in the wound bed at this time. PDGF initiates the chemotaxis of macrophages, neutrophils, smooth muscle cells and fibroblasts. TGF- β attracts macrophages and stimulates them to produce additional cytokines. Cytokines, such as interleukin (IL)-1 α and IL-1 β , recruit inflammatory cells and fibroblasts to the site of injury, causing inflammation and vasodilatation, which increases blood vessel permeability, and allows easy passage of fluid and phagocytes. PDGF is released by the platelets, stimulating the growth of blood vessels and new structural tissue (Morrison, 1997).

Approximately 24 hours after injury, neutrophils and macrophages begin to remove nonviable tissue and bacteria from the wound through the release of enzymes and phagocytosis (Harding, Morris & Patel, 2002). In addition to removing nonviable tissue from the wound bed, macrophages and neutrophils have been shown to express several pro-inflammatory cytokines that serve as some of the earliest signals to activate fibroblasts and keratinocytes (Hubner et al., 1996; Mehendale & Martin; 2001, Kindlen & Morrison; 1997). This acute inflammatory phase typically last between 24 and 48 hours but can potentially persist for up to 2 weeks if necrotic tissue or high bacterial loads remain in the wound (Doughty & Sparks-Defriese, 2007; Li, Chen, & Kirsner, 2007).

At approximately 48 hours, fixed tissue monocytes are activated to become wound macrophages and are essential for removing nonfunctional host cells, bacteria-filled neutrophils, damaged wound matrix, foreign debris, and any remaining bacteria. These activated cells release PDGF and TGF- β , attracting smooth muscle cells and fibroblasts (Diegelmann & Evans, 2004).

Over approximately 2 days to 3 weeks, the proliferative phase begins. TGF- β becomes an important regulator of fibroblast function during this phase (Diegelmann & Evans, 2004). Fibroblasts and other cell types begin to lay down the ground substances and collagen fibers in the site of injury. Various chemical mediators such as PDGF stimulate angiogenesis, which is marked by the formation of granulation tissue consisting of new capillary loops in a matrix of collagen and ground substance. Keratinocytes at the wound edges and from the remnants of hair follicles, sebaceous glands and sweat glands divide and begin to migrate, laying down new epithelium. Wound contraction occurs as a result of actions of the myofibroblasts.

Over the next 3 weeks to 2 years, the final phase of wound healing takes place. In the maturation or remodeling phase, type III collagen is gradually replaced with type I. Tensile strength increases as the collagen fibers reorganize. Healed wounds result in a scar, which differs somewhat from the original tissue and has approximately 80% of the original tensile strength (Kindlen & Morrison, 1997).

Cytokines, specifically PDGF, tumor necrosis factor (TNF- α), interferon-gamma (IFN- γ), various interleukins (IL- 1 α , IL-1 β , IL-6, IL-8), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and transforming growth factor-beta (TGF- β) mediate many of the complex interactions involved in wound healing. Wounds typically progress in a predictable, timely manner. When the normal phases of wound healing are interrupted, chronic wounds develop, leading to increased risk of infection, increased hospital stays, and decreased quality of life. Chronic wounds account for a significant amount of healthcare spending in the United States, estimated to be \$5 to \$9 billion each year (Mandracchi, John, & Sanders, 2001).

Stress and Wound Healing

Psychological stress has been shown to adversely affect the normal wound healing process through its impact on cellular immunity. Cellular immunity has an important role in the regulation of wound healing through the production and regulation of pro-inflammatory and anti-inflammatory cytokines. Numerous studies have shown the relationship of psychological stress and wound healing. One such study examined the effects of caregiver stress on wound healing, demonstrating that caregivers of individuals with dementia, who reported significantly more stress than did controls, took longer to heal a 3.5mm punch biopsy wound (Kiecolt-Glaser, 1995). Ebrecht et al. (2003) investigated the association between perceived stress and wound healing by inflicting 4mm punch biopsy wounds on nonsmoking male subjects and then looked at time to healed wound. A negative correlation between speed of wound healing and both the Perceived Stress Scale and the General Health Questionnaire were found. Morning cortisol levels were highest in those individual whose wounds were the slowest to heal. Glaser et al. (1999) found that women with higher perceived stress

scores had higher levels of salivary cortisol and significantly lower levels of IL-1 and IL-8 at the wound site. In patients undergoing routine inguinal hernia repair, greater preoperative perceived stress significantly predicted lower levels of IL-1 in the wound bed. Patients who reported higher levels of worry about the operation had lower levels of matrix metalloproteinase-9 in the wound bed. Preoperative worry was also associated with greater postoperative pain, poorer self-rating of recovery and a prolonged recovery time (Broadbent, 2003). Rose et al (2001) point out that stress can negatively impact sleep, leading to disturbed sleep patterns and a reduction in growth hormones, which may down-regulate the tissue repair response. Although many factors play a role in whether or not a wound heals, such as proper nutrition, underlying health conditions and appropriate care, it is clear that cytokines play a crucial role as well. If dysregulation of the various cytokines occurs, a potential disruption of normal wound healing results, leading to delayed healing and increased risk of infection.

Stress and Breast Cancer

According to the American Cancer Society, breast cancer is the most common malignancy diagnosis in women. There were approximately 270,000 new cases of breast cancer in 2005. Approximately 62,000 deaths were due to breast cancer in that same year (American Cancer Society, 2006). In 2004, approximately 62,000 women underwent mastectomy with some form of reconstruction (National Women's Health Resource Center, 2005). The diagnosis of breast cancer is identified as a major life stressor (Kiecolt-Glaser & Glaser, 1994). Some studies indicate up to 80% of these women report significant stress during their initial treatment (Irvine, Vincent, Graydon & Bubela, 1998). While a study by Ananian et al. (2004) showed that 83% of women choose immediate breast reconstruction, a study by Roth, Lowery, Davis and Wilkins (2005) found that there was a relatively higher incidence of psychosocial impairment and functional disability in women seeking immediate reconstruction following mastectomy. Studies have looked at psychological factors in women with breast cancer and how such factors impact satisfaction with their reconstruction and quality of life (Roth et al., 2005), yet little is known about the effect of pre- and post-operative stress associated with mastectomy and reconstruction and its potential affects on wound healing.

Surgical wound complications such as infection, delayed closure and wound dehiscence are associated with longer hospital stays, decreased quality of life, and increases in morbidity and mortality. Wounds that fail to progress through the orderly healing process and convert to chronic wounds greatly increase cost of care and lead to increases in morbidity and mortality.

By understanding the role of cytokines in the wound environment, we may better understand the complex chemical interactions and the chemical mediators of healing in the wound. By gaining such understanding, we may further develop technologies and interventions that impact the wound environment at the cellular and biochemical levels, leading to improved healing of both acute and the chronic wounds. Examination of wound fluids has the potential to develop into monitoring tools, to serve as markers for certain

critical events in the tissues, and subsequently offer diagnostic and prognostic information as well as offer an opportunity to more effectively evaluate wound healing therapies. For example, in a study evaluating wound fluid from 47 patients who had undergone mastectomy, one group found that surgical wounds that later developed an infection (11%) had significantly lower PDGF and ECGF on post-operative day 1 than the non-infected wounds ($p < 0.05$). They also found that 62% of the patients who developed a seroma had significantly lower levels of bFGF than those patients who did not develop a seroma ($p < 0.05$) (Baker, Kumar, Melling, Whetter & Leaper, 2008). Tarlton, Vickery, Leaper and Baily (1997) found that a decline in MMP-9 levels between 24 to 48 hours post-op was a predictor of infection. In another study, Chow, Loo, Yuen and Cheng found that high levels of IL-4 in wound fluid on post-operative day 1 or high levels of IL-6 on post-operative day 2 in women who had undergone mastectomy was associated with risk of flap necrosis.

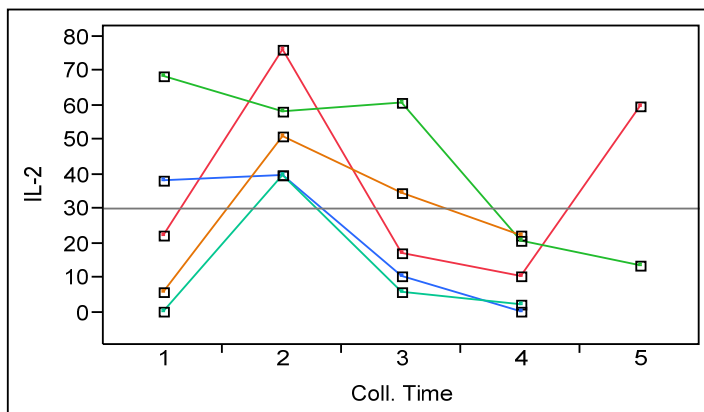
The goal of this pilot study project is to examine the relationships among pre- and post-operative psychological stress experienced by women who are undergoing mastectomy with immediate reconstruction and wound healing, specifically the chemical mediators of wound healing in the local wound environment.

VIII. PRELIMINARY PROGRESS/DATA REPORT**If available.**

A feasibility study was successfully conducted with 5 subjects to evaluate feasibility of the project and to refine the process of consent, participant enrollment, data collection and analysis. Subjects were willing to enroll and participate fully and showed great interest in the project. There was 100% return rate on psychological measures was observed in the feasibility study. Each of the psychological measures obtained showed an appropriate level of variability thus appeared promising in regard to their ability to correlate with biological measures. Wound fluid was easily obtainable, transported and stored. There were no problems associated with retrieving adequate volume for the 27 Bioplex analysis. Trends in cytokines were detected and were supported by the research reported above. For example, the plot of IL-6 (see figure 1) illustrates a rapid rise from time 1 (24 hours) to time 2 (48 hours) and finally back to time 1 levels at time 4 (72 hours). Interesting trends were observed in nearly all the cytokines. However, IL-6 was chosen as a surrogate for the other 26 cytokines because IL-6 is most commonly cited in the literature. The trends seen in the feasibility data support our hypothesis and thus indicate the need for further study with a larger sample size.

Figure 1

Fit Y by X Group
Oneway Analysis of IL-2 By Coll. Time



IX. RESEARCH METHOD AND DESIGN

Include a brief description of the project design including the setting in which the research will be conducted and procedures. If applicable, include a description of procedures being performed already for diagnostic or treatment purposes.

This pilot study will prospectively evaluate 20 women with breast cancer who are having total or modified radical mastectomies with immediate or delayed reconstruction with autologous abdominal tissue. Although our sample size is small, previous studies (Takamiya, Fujita, Saigusa & Aoki, 2007) have demonstrated that meaningful cytokine patterns can be found with a sample as small as 5. Data collected will include demographics, cancer type and stage, length of surgery, intra-operative complications, medical co-morbidities and medications, pain levels and pain medications used, and previous treatment with chemotherapy.

Methods

After obtaining informed consent the following procedures will be used to obtain study data:

Patients usually come to the clinic approximately 7-10 days before their scheduled surgery for a preoperative history and physical exam. The stress questionnaires will be administered at that time. The 10-item Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983), 100mm Visual Analog Scale measuring stress, and the 22-item Impact of Events Scale-Revised (Creamer, Bell & Failla, 2003) will be administered at that time to evaluate preoperative perceived stress levels. Two cc (about 1 tsp) of blood will be collected in concert with pre-operative blood work to evaluate peripheral blood cytokines.

Wound fluid, collected by the student investigator, will be removed from the tubing of the Jackson-Pratt drain, which is already in place post-operatively and is a routine method for post-operative serous fluid management in the surgical site. Fluid will be collected at 24, 48, 72, and 96 hours post-operatively from the Jackson-Pratt tubing and stored at -70 C in the VCU Center for Biobehavioral Clinical Research until samples have been collected from all participants. At the 48-hour collection, an additional IES-R and the stress visual analog scale will be administered to the patient. If the patient is too tired or sedated to read the form, the student investigator will read the form and record answers for the study subject. An additional 2 cc of blood will be collected for peripheral blood cytokine analysis at this time as well.

Levels of cytokines in the wound fluid and blood will be determined using a Bio-Plex Magnetic Bead Array System (Bio-Rad®) with the 27-plex cytokine detection kit according to the kit manufacturer's protocol. Assays will be performed in the VCU School of Nursing CBCR laboratory; we have extensive experience with measurement of analytes from human samples. The Bio-Plex assay combines fluorescent flow cytometry and ELISA technology, providing simultaneous quantitation of each of the 27 cytokines using an analyte quantity as small as 12 µl. The manufacturer reports that the assay accurately measures cytokine values in the range of 1-2,500 pg/ml (well within the limits of detection for this project), is precise (intra-assay CV < 10%, inter-

assay CV < 15%), and shows less than 1% cross-reactivity among cytokines or with other molecules.

Demographics, medications and comorbidities will be recorded at pre-op visit; comorbidities and cancer type/stage verified postoperatively. The student researcher will directly ask the patient to rate her pain at the time of each fluid collection. Pain will be rated on scale of 0-10, with 0 being no pain and 10 being the worst pain. Subjects will be followed weekly to biweekly as part of their routine post surgical care. Surgical complications such as the development of seroma, flap necrosis or infection, will be followed during this time and will be documented if they occur. Additionally, we will monitor total length of time the drains were present.

X. PLAN FOR CONTROL OF INVESTIGATIONAL DRUGS, BIOLOGICS, AND DEVICES.

For investigational drugs and biologics: IF IDS is not being used, attach the IDS confirmation of receipt of the management plan.. See item #11 on Initial Review form.

For investigational and humanitarian use devices (HUDs): Describe your plans for the control of investigational devices and HUDs including: (1) how you will maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the return to the sponsor or alternative disposition of unused product(s); (2) plan for storing the investigational product(s)/ HUD as specified by the sponsor (if any) and in accordance with applicable regulatory requirements; (3) plan for ensuring that the investigational product(s)/HUDs are used only in accordance with the approved protocol; and (4) how you will ensure that each subject understands the correct use of the investigational product(s)/HUDs (if applicable) and check that each subject is following the instructions properly (on an ongoing basis).

N/A

XI. DATA ANALYSIS PLAN**For investigator-initiated studies.**

All demographic data will be summarized using descriptive statistics (i.e. means and variance for continuous data and frequencies for the categorical data). Using profile plots (commonly referred to as spaghetti plots), the biologic data will be illustrated over time both within and across the cytokines. These plots will be visually examined for similarities. It is anticipated that the observed trends will suggest groupings of the cytokines and, as a consequence, suggest ways of reducing the dimensionality of the data (e.g., the development of a composite score using the factor loading from factor analysis).

The levels of perceived stress will be examined descriptively with the intention of detecting associative trends with the biologic data. We will also look for correspondence of peripheral blood cytokines and wound fluid cytokines using the Pearson's correlation and examine correlations between pre- and post-operative stress and peripheral blood and wound fluid cytokines. It has been the experience of the investigators that the cytokine data are not normally distributed. Thus, it is anticipated that the log transformed cytokines will be used for calculation of the correlations.

XII. DATA AND SAFETY MONITORING

- If the research involves greater than minimal risk and there is no provision made for data and safety monitoring by any sponsor, include a data and safety-monitoring plan that is suitable for the level of risk to be faced by subjects and the nature of the research involved.
- If the research involves greater than minimal risk, and there is a provision made for data and safety monitoring by any sponsor, describe the sponsor's plan.
- If you are serving as a Sponsor-Investigator, identify the Contract Research Organization (CRO) that you will be using and describe the provisions made for data and safety monitoring by the CRO. Guidance on additional requirements for Sponsor-Investigators is available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#X-2.htm

The pilot study involves the administration of two questionnaires, stress rating on a visual analog scale, collection of fluid from drains previously inserted for medical purposes, and the collection of blood. The study involves no intervention and no interference with the placement of the drain. The first peripheral blood draw will take place at the time of preoperative blood work. The second peripheral blood draw will be performed by the student researcher who is a licensed nurse practitioner and is fully trained in venipuncture procedures.

The investigators will be the only individuals allowed to review the data with patient identifiers in place. Each subject will be assigned a study number, which will be the only link to identifying information. No identifying information will be entered into the electronic database. The master list of patient names, medical record numbers and study numbers will be kept in a secure place and destroyed after the data collection is

completed.

XIII. MULTI-CENTER STUDIES

If VCU is the lead site in a multi-center project or the VCU PI is the lead investigator in a multi-center project, describe the plan for management of information that may be relevant to the protection of subjects, such as reporting of unexpected problems, project modifications, and interim results.

N/A

XIV. INVOLVEMENT OF NON-VCU INSTITUTIONS/SITES (DOMESTIC AND FOREIGN)

1. Provide the following information for each non-VCU institution/site (domestic and foreign) that has agreed to participate:

- Name of institution/site
- Contact information for institution/site

N/A

2. For each institution, indicate whether or not it is “engaged” in the research (see OHRP’s guidance on “Engagement of Institutions in Research” at <http://www.hhs.gov/ohrp/humansubjects/assurance/engage.htm>.)

N/A

3. Provide a description of each institution’s role (whether engaged or not) in the human subjects research, adequacy of the facility (in order to ensure human subject safety in the case of an unanticipated emergency), responsibilities of its agents/employees, and oversight that you will be providing in order to ensure adequate and ongoing protection of the human subjects. You should only identify institutions that have agreed to participate. If additional institutions agree to participate at a later time, they must be added by amendment to the protocol.

N/A

4. For each institution that is “engaged” provide an OHRP Federalwide Assurance (FWA) # if: (1) the research is not exempt, AND (2) the research involves a DIRECT FEDERAL award made to VCU (or application for such).

NOTE: Additional guidance at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XVII-6.htm, and http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XVII-11.htm.

N/A

XV. INVOLVEMENT OF INDEPENDENT INVESTIGATORS

INDEPENDENT INVESTIGATOR: an individual who is acting independently and not acting as an agent or employee of

any institution or facility while carrying out his or her duties in the research protocol. Additional guidance at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XVII-15.htm.

ENGAGEMENT IN RESEARCH: An independent investigator becomes "engaged" in human subjects research when he/she (i) intervenes or interacts with living individuals for research purposes; or (ii) obtains individually identifiable private information for research purposes [45 CFR 46.102(d)-(f)]. See OHRP's guidance on "Engagement of Institutions in Research" at <http://www.hhs.gov/ohrp/humansubjects/assurance/engage.htm>.

1. Provide a list of independent investigators.
2. For each independent investigator indicate whether or not he/she is "engaged" or "not engaged" in the research
3. For each independent investigator who is "engaged": (1) describe his/her role with human subjects/identifiable human data, AND (2) describe YOUR oversight of his/her involvement.

N/A

NOTE: If an independent investigator is "engaged," and the research is (1) not exempt AND (2) involves a DIRECT FEDERAL award made to VCU (or application for such), the independent investigator must sign a formal written agreement with VCU certifying terms for the protection of human subjects. For an agreement to be approved: (1) the PI must directly supervise all of the research activities, (2) agreement must follow the ORSP template, (3) IRB must agree to the involvement of the independent investigator, AND (4) agreement must be in effect prior to final IRB approval.

XVI. HUMAN SUBJECTS INSTRUCTIONS (Be sure to use the sub-headings under A-I)

ALL sections of the Human Subjects Instructions must be completed with the exception of the section entitled "Special Consent Provisions." Complete that section if applicable.

A. DESCRIPTION

Provide a detailed description of the proposed involvement of human subjects or their private identifiable data in the work.

A convenience sample of 20 women with breast cancer, age 21-65, who will be having immediate or delayed reconstruction after their total or modified radical mastectomy with an abdominally based autologous tissue will be recruited for this study. Following explanation of the study and fully informed, documented consent, participants will be enrolled and demographic information will be collected. Participants will be asked to complete the 10-item Perceived Stress Scale (PSS), the Impact of Event Scale Revised (IES-R) and a visual stress an . During this visit, patients are usually sent to the lab to have their pre-operative blood work drawn. An additional tube of blood will be collected for our research to analyze peripheral cytokines. Approximately 24 hours after their surgery, the second Perceived Stress Scale will be administered. If the study participant is too weak or sedated to read the survey, then the student researcher will read the questionnaire. At this time, the student researcher will also be obtaining a second blood draw.

B. SUBJECT POPULATION

Describe the subject population in terms of sex, race, ethnicity, age, etc., and your access to the population that will allow recruitment of the necessary number of participants. Identify the criteria for inclusion or exclusion of any subpopulation and include a justification for any exclusion. Explain the rationale for the involvement of special cases of subjects, such as children, pregnant women, human fetuses, neonates, prisoners or others who are likely to be

vulnerable. If you plan to allow for the enrollment of Wards of the State (or any other agency, institution, or entity), you must specifically request their inclusion and follow guidance on Wards and Emancipated Minors in the VCU IRB Written Policies and Procedures (specifically WPP#: XV-3) available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XV-3.htm.

The target population will be women referred to the VCUHS Plastic and Reconstructive Surgery practice for breast reconstruction following total or modified radical mastectomy for a diagnosis of breast cancer. All women who are considering autologous abdominal tissue reconstruction will be potential candidates in accordance with the study inclusion and exclusion criteria. Prisoners will be excluded, as their circumstances involving transportation may be extenuating.

For consideration of an autologous abdominal tissue reconstruction, a multitude of factors are taken into consideration to identify suitable appropriate surgical candidates. The length and complexity of these reconstructive surgical procedures requires that patients be evaluated based on risk factors proposed by Hartrampf (1991) which include psychological stability, diabetes, vascular disease, healthy weight, smoking history, past abdominal surgeries, pulmonary disease and cardiac disease. Essentially, women who are considered good surgical candidates and selected by the surgeon for the procedure are in good health other than their cancer diagnosis.

Inclusion criteria for this pilot study will include females aged 21 – 65, who have been diagnosed with breast cancer and are undergoing autologous abdominal tissue reconstruction following their mastectomies.

Exclusion criteria include

1. Patients on immunosuppressive medications and those women who have had a hysterectomy or oophorectomy or will be undergoing hysterectomy and/or oophorectomy at the time of mastectomy. We have excluded these participants to control hormonal effects this type of surgery may cause.
2. Pregnancy
3. Past medical history of autoimmune disorders

We plan to enroll 20 participants. Enrollment will occur through convenience sampling using the above criteria. Study sampling will not interfere with required and scheduled care of the participant. Participants may withdraw from the study at any time. This will not impact the care of the participant in any way.

C. RESEARCH MATERIAL

Identify the sources of research material obtained from individually identifiable living human subjects in the form of specimens, records, or data. Indicate whether the material or data will be obtained specifically for research purposes or whether use will be made of existing specimens, records, or data.

Research material includes wound fluid samples that would otherwise be discarded, blood samples, demographic information, information regarding immediate operative and post-operative course, and information obtained from the stress questionnaires and analog scales. These materials will be obtained for research purposes only.

D. RECRUITMENT PLAN

Describe in detail your plans for the recruitment of subjects including: (1) how potential subjects will be identified (e.g., school personnel, health care professionals, etc), (2) how you will get the names and contact information for potential subjects, and (3) who will make initial contact with these individuals (if relevant) and how that contact will be done. If you plan to involve special cases of subjects, such as children, pregnant women, human fetuses, neonates, prisoners or others who are likely to be vulnerable, describe any special recruitment procedures for these populations.

Following IRB approval, subjects will be recruited prospectively as they present to the Plastic and Reconstructive Surgery department for their pre-operative appointment. These patients will have already had their first consultation with the plastic surgeon and the type of reconstruction will have already been decided upon and scheduled. The plastic surgeons will offer the patient the opportunity to participate in this pilot study and then the study staff will approach the patient if the patient is interested in participating.

E. POTENTIAL RISKS

Describe potential risks whether physical, psychological, social, legal, or other and assess their likelihood and seriousness. Where appropriate, describe alternative treatments and procedures that might be advantageous to the subjects.

This pilot study presents no more than minimal risk to the study participants. Participants may be inconvenienced by providing demographic information or completing the second PSS. Risks associated with venipuncture include mild pain and/or bruising at the site. Another potential risk to the subjects is breach of confidentiality.

F. RISK REDUCTION

Describe the procedures for protecting against or minimizing potential risk. Where appropriate, discuss provisions for ensuring necessary medical or professional intervention in the event of adverse events to the subjects. Also, where appropriate, describe the provisions for monitoring the data collected to ensure the safety of subjects.

This pilot study presents no more than minimal risk to study participants. Written informed consent will be required for enrollment and participation in this study. The student investigator will review the study fully with potential participants and answer any questions they may have.

Access to wound fluid will be through a Jackson-Pratt drain, which is standard of care in these patients. The drain will not be kept in longer than medically necessary, as decided by the plastic surgeon who performed the surgery. Therefore, risk associated with collection of drain fluid is minimal.

Risk associated with venipuncture is reduced by collecting the first blood sample during routine blood work evaluation and by use of sterile procedures and universal precautions when the experienced, student researcher draws the second, post-operative blood sample.

A potential risk to the subjects is breach of confidentiality. In order to minimize such risk, each subject will be assigned a study number, and no other identifying information will be recorded. A master list of medical record numbers and study numbers will be available only to study staff for purposes of chart identification and will be kept in a secure, locked file cabinet when not in use. Once all of the data have been collected, this master list will be destroyed.

G. ADDITIONAL SAFEGUARDS IF ANY PARTICIPANTS WILL BE VULNERABLE

Describe any additional safeguards to protect the rights and welfare of participants if you plan to involve special cases of subjects, such as children, pregnant women, human fetuses, neonates, prisoners or others who are likely to be vulnerable. Safeguards to protect the rights and welfare of participants might relate to Inclusion/Exclusion Criteria: ("Adults with moderate to severe cognitive impairment will be excluded." "Children must have diabetes. No normal controls who are children will be used.") Consent: ("Participants must have an adult care giver who agrees to the participant taking part in the research and will make sure the participant complies with research procedures." "Adults must be able to assent. Any dissent by the participant will end the research procedures.") Benefit: ("Individuals who have not shown benefit to this type of drug in the past will be excluded.").

N/A

H. CONFIDENTIALITY

Describe how the confidentiality of data collected as part of this project will be protected including pre-screening data (e.g., physical controls on the data; access controls to the data; coding of data; legal controls, such as a Federal Certificate of Confidentiality; statistical methods; or reporting methods).

The investigators will be the only individuals allowed to review the data with patient identifiers in place. All data will be maintained by the student investigator and identities protected. Unidentifiable coding procedures consist of each subject being assigned a study number, which will be the only identifying information collected and recorded in the electronic database. The master list of patient names, medical record numbers and study numbers will be kept in a secure place and destroyed after the data collection is completed. All survey and laboratory data will be coded.

I. PRIVACY

Describe how the privacy interests of subjects will be protected where privacy refers to persons and their interests in controlling access to themselves, and assess their likely effectiveness. Identify what steps you will take for subjects to be comfortable: (1) in the research setting and (2) with the information being sought and the way it is sought.

Study participants will be given time to complete surveys in a private setting. The investigators will be the only individual allowed to review the data with patient identifiers in place. Each subject will be assigned a study number, which will be the only identifying information collected and recorded in the electronic database. The master list of patient names, medical record numbers and study numbers will be kept in a secure place and destroyed after the data collection is completed.

J. RISK/BENEFIT

Discuss why the risks to subjects are reasonable in relation to the anticipated benefits to subjects and in relation to the importance of the knowledge that may reasonably be expected to result. If a test article (investigational new drug, device, or biologic) is involved, name the test article and supply the FDA approval letter.

There is no direct benefit to the participant although there is potential for future benefit to others. Risk to the participant is minimal. The wound fluid of interest will be withdrawn through a drain previously placed for medical purposes; this fluid is usually measured for volume and immediately discarded. Once data are collected, all personal identifying information will be destroyed.

K. COMPENSATION PLAN

Compensation for subjects (if applicable) should be described, including possible total compensation, any proposed bonus, and any proposed reductions or penalties for not completing the project.

No compensation will be offered.

L. CONSENT ISSUES**1. CONSENT PROCESS**

Indicate who will be asked to provide consent/assent, who will obtain consent/assent, what language (e.g., English, Spanish) will be used by those obtaining consent/assent, where and when will consent/assent be obtained, what steps will be taken to minimize the possibility of coercion or undue influence, and how much time will subjects be afforded to make a decision to participate.

Following IRB approval and prior to any data collection, documented informed consent will be obtained by the student investigator only from those patients who agree to be in the study. Patients will not be approached by the investigator until the surgeon has discussed the study with the patient. If the patient agrees to learn more about the study, then the investigator will review the consent information with the patient and answer any questions. Ideally, this first step will occur at the consultation visit, if at that time an autologous abdominal tissue reconstruction is decided upon. If the potential participant needs more time, she may take the consent information home and call the PI or student investigator with any questions or concerns. Fully informed consent will be obtained prior to surgery, either at the consultation visit or the pre-operative visit. No additional travel will be required above what is required for routine medical care.

2. SPECIAL CONSENT PROVISIONS

If some or all subjects will be cognitively impaired, or have language/hearing difficulties, describe how capacity for consent will be determined. Please consider using the VCU Informed Consent Evaluation Instrument available at <http://www.research.vcu.edu/irb/guidance.htm>. If you anticipate the need to obtain informed consent from legally authorized representatives (LARs), please describe how you will identify an appropriate representative and ensure that their consent is obtained. Guidance on LAR is available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XI-3.htm.

N/A

3. If request is being made to WAIVE SOME OR ALL ELEMENTS OF INFORMED CONSENT FROM SUBJECTS OR PERMISSION FROM PARENTS, explain why: (1) the research involves no more than minimal risk to the subjects, (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects, (3) the research could not practicably be carried out without the waiver or alteration; AND (4) whether or not subjects will be debriefed after their participation. Guidance is available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XI-1.htm. **NOTE: Waiver is not allowed for FDA-regulated research unless it meets FDA requirements for Waiver of Consent for Emergency Research (see below).**

N/A

4. If request is being made to **WAIVE DOCUMENTATION OF CONSENT**, provide a justification for waiver based on one of the following two elements **AND** include a description of the information that will be provided to participants: (1) the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Subject will be asked whether they want documentation linking them with the research, and each subject's wishes will govern; or (2) the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context. Guidance is available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XI-2.htm

N/A

5. If applicable, explain the **ASSENT PROCESS** for children or decisionally impaired subjects. Describe the procedures, if any, for re-consenting children upon attainment of adulthood. Describe procedures, if any, for consenting subjects who are no longer decisionally impaired. Guidance is available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XV-2.htm, and http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XVII-7.htm.

N/A

6. If request is being made to **WAIVE THE REQUIREMENT TO OBTAIN ASSENT** from children age 7 or higher, or decisionally impaired subjects, explain why: (1) why some or all of the individuals age 7 or higher will not be capable of providing assent based on their developmental status or impact of illness; (2) the research holds out a prospect of direct benefit not available outside of the research; **AND/OR** (3) [a] the research involves no more than minimal risk to the subjects, [b] the waiver or alteration will not adversely affect the rights and welfare of the subjects, [c] the research could not practicably be carried out without the waiver or alteration; **AND** [d] whether or not subjects will be debriefed after their participation. Guidance is available at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XV-2.htm

N/A

7. If request is being made to waive consent for emergency research, see guidance at http://www.research.vcu.edu/irb/wpp/flash/wpp_guide.htm#XVII-16.htm.

N/A

8. If applicable, address the following issues related to **GENETIC TESTING**:

No genetic testing is involved in this research protocol

a. **FUTURE CONTACT CONCERNING FURTHER GENETIC TESTING RESEARCH**

Describe the circumstances under which the subject might be contacted in the future concerning further participation in this or related genetic testing research.

N/A

b. FUTURE CONTACT CONCERNING GENETIC TESTING RESULTS

If planned or possible future genetic testing results are unlikely to have clinical implications, then a statement that the results will not be made available to subjects may be appropriate. If results might be of clinical significance, then describe the circumstances and procedures by which subjects would receive results. Describe how subjects might access genetic counseling for assistance in understanding the implications of genetic testing results, and whether this might involve costs to subjects. Investigators should be aware that federal regulations, in general, require that testing results used in clinical management must have been obtained in a CLIA-certified laboratory.

N/A

c. WITHDRAWAL OF GENETIC TESTING CONSENT

Describe whether and how subjects might, in the future, request to have test results and/or samples withdrawn in order to prevent further analysis, reporting, and/or testing.

N/A

d. GENETIC TESTING INVOLVING CHILDREN OR DECISIONALLY IMPAIRED SUBJECTS

Describe procedures, if any, for consenting children upon the attainment of adulthood. Describe procedures, if any, for consenting subjects who are no longer decisionally impaired.

N/A

e. CONFIDENTIALITY

Describe the extent to which genetic testing results will remain confidential and special precautions, if any, to protect confidentiality.

N/A

Perceived Stress and Surgical Wound Cytokine Patterns

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Abstract

This was a pilot study to examine pre- and post-operative stress experienced by women who were undergoing autologous breast reconstruction and how stress might impact wound healing, specifically examining cytokines and other the chemical mediators in the wound environment. A non-experimental descriptive design over time was utilized. Participants were women who were undergoing autologous abdominal breast reconstruction for breast cancer ($N=20$). Data were collected pre-operatively and at 24, 48, 72 and 96 hours post surgery. Complications were monitored intra-operatively and up to 30 days post surgery. Psychological stress was measured with the 10-item Perceived Stress Scale (PSS), the Impact of Events Scale-Revised (IES-R), and a 100mm Visual Analogue Scale (VAS). Cytokines were assayed using the 27-plex kit with a Bio-Plex Plus®. While breast cancer is considered a stressor, in this sample of women, scores of the PSS, IES-R and VAS showed that in fact these participants experienced low levels of psychological stress. All measured biochemical mediators in serum and wound fluid were detected and trends were identified. IL-1ra, IL-6, IL-8, G-CSF, IP-10, MCP1, MIP-1b, RANTES and VEGF were present in the highest concentrations. Significant changes in levels of cytokines in wound fluid were observed in IL-1 β , IL-2, IL-5, IL-6, IL-8, IL-9, IL-10, IL-17, FGF-basic, G-CSF, MIP-1a, PDGF-bb, MIP1b, RANTES, and TNF- α . The remaining cytokine concentrations remained stable over time. These findings suggest that although these women were not experiencing high levels of stress, meaningful cytokine patterns were detected.

Keywords: psychological stress, wound healing, cytokine, growth factor

Introduction

Research has shown a strong relationship between psychological stress and health. Thought of as a human response to a specific stimulus or “stressor”, stress can initiate a cascade of events leading to a variety of detrimental effects on health. One such negative impact is that on wound healing. The mechanism by which stress is thought to exert negative impact on wound healing is through its effects on cellular immunity, leading to a disruption of the wound healing process. When normal wound healing is interrupted, chronic wounds develop, leading to increased risk of infection, increased hospital stays, and decreased quality of life. Numerous studies have shown that psychological stress may slow wound healing, but specific mechanisms remain uncertain. “Analysis of wound fluid provides an opportunity to potentially connect the mechanism of psychological stress to cellular mechanisms in the local wound site” (Lucas, 2011, p. 80). The purpose of this research was to examine the relationships among pre- and post-operative psychological stress experienced by women who were undergoing autologous abdominal reconstruction for mastectomy. Specifically, the biochemical mediators of wound healing in the local wound environment were examined over time.

Background

The Stress Response and Wound Healing

Psychological stress, once experienced by an individual, results in neuroendocrine signals being transmitted from the brain leading to changes in the immune system. Cellular immunity has an important role in the regulation of wound healing through the production and regulation of pro-inflammatory and anti-inflammatory cytokines. Stress affects neuroendocrine functioning via two pathways, the hypothalamic-pituitary-adrenal

(HPA) axis of the central nervous system and the sympathetico-adreno-medullary (SAM) axis of the sympathetic nervous system. When we encounter a stressful event, there is simultaneous activation of the HPA axis and the SAM axis.

Activation of the SAM axis causes acetylcholine to be secreted from the adrenal medulla. This leads to release of the catecholamines norepinephrine and epinephrine into the systemic blood supply. This activation is responsible for the classic “Fight or Flight” response, such as increased heart rate, increased blood flow to the skeletal muscles, and increased glucose metabolism. These catecholamines also are responsible for activating the inflammatory response (Glaser & Kiecolt-Glaser, 2005; Sorrells & Sapolsky, 2007; Vileikyte, 2007; Yang & Glaser, 2002).

When the HPA system is activated by stress, corticotropin-releasing hormone (CRH) is released from the hypothalamus. CRH then stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary into systemic circulation, which in turn triggers release of the glucocorticoid cortisol from the adrenal cortex. Elevated levels of cortisol suppress the immune system in a variety of ways. Cortisol inhibits the migration of leukocytes to a site of infection by decreasing capillary permeability and inhibiting chemotaxis. Cortisol has also been shown to stabilize lysosomal membranes thus inhibiting release of their infection-fighting proteolytic enzymes. Cortisol decreases fibroblast proliferation and function at the site of injury as well as inhibits the release of inflammatory substrates such as histamine and prostaglandins. Importantly, cortisol inhibits production of certain cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF) (Boyapati & Wang, 2007; Glaser & Kiecolt-Glaser, 2005).

As recently reviewed by Lucas (2011), wound healing is regulated by cellular processes controlled by the immune system. The complex and orderly process of normal cutaneous wound healing occurs in overlapping phases and depends upon multiple interactions among various biochemical mediators and cell types. Successful wound healing is highly dependent on an orderly progression of the first phase, the inflammatory phase. Within seconds of injury, the inflammatory phase is initiated and can last anywhere from 2 to 7 days. Platelets are activated when blood vessels are disrupted and clotting factors are released. Vasoconstriction and platelet aggregation lead to the formation of a provisional matrix for cellular migration of neutrophils and macrophages into the wounded area to begin to remove bacteria from the wound and initiate tissue repair (Christian, Graham, Padgett, Glaser, & Kiecolt-Glaser, 2006; Doughty & Sparks-Defries; 2007; Li, Chen, & Kirsner, 2007; Mehendale & Martin, 2001). Inflammatory mediators such as cytokines play a crucial role in attracting phagocytes to the wound bed and orchestrating the production and release of growth factors and matrix metalloproteinase (MMP) enzymes crucial for collagen organization. These early biochemical mediators also play a role in recruiting cells and growth factors needed later for tissue regeneration (Monaco & Lawrence, 2003; Singer & Clark, 1999).

Stress and Breast Cancer

According to the American Cancer Society, breast cancer is the most common malignancy diagnosis in women. In the United States, there were approximately 284,520 new cases of breast cancer in 2011. Approximately 39,520 deaths were attributed to breast cancer in that same year (American Cancer Society, 2011). In 2010, approximately 93,000 women underwent mastectomy with some form of reconstruction (American

Society of Plastic Surgeons, 2011). Ananian et al. (2004) showed that 83% of women choose immediate breast reconstruction following mastectomy. However, Roth, Lowery, Davis, and Wilkins (2005) documented a relatively higher incidence of psychosocial impairment and functional disability in women who pursue immediate reconstruction following mastectomy. Investigators have looked at psychological factors in women with breast cancer and how such factors impact satisfaction with their reconstruction and quality of life (Roth et al., 2005), yet little is known about the effects of pre- and post-operative stress associated with mastectomy and reconstruction on the process and outcomes of wound healing.

Surgical wound complications such as infection, delayed closure, and wound dehiscence are associated with longer hospital stays, decreased quality of life, and increases in morbidity and mortality (Zhan & Miller, 2003). Wounds that fail to progress through the orderly healing process and convert to chronic wounds even more significantly increase cost of care, decrease quality of life, and lead to increases in morbidity and mortality (Sen et al., 2009; Shukla et al., 2008).

By regulating chemotaxis and cellular proliferation, cytokines, chemokines, and growth factors, which for the purpose of this paper will be referred to as biochemical mediators, are involved in the initiation, control, and termination of the cellular processes that occur at each phase of wound healing. Each biochemical mediator functions in various ways, either alone, synergistically with another, or overlapping with another, to accomplish healing (Baker, El-Gaddal, Aitken, & Leaper, 2003). By understanding the role of these biochemical mediators in the wound environment, we may better understand the complex biochemical interactions and mediators of healing in the wound. Given such

understanding, we may further develop technologies and interventions that impact the wound environment at the cellular and biochemical levels, leading to improved healing of both acute and chronic wounds.

Examination of wound fluids has the potential to aid in developing monitoring tools, to identify markers for certain critical events in the tissues, and subsequently to offer diagnostic and prognostic information as well as opportunities to more effectively evaluate wound healing therapies. For example, in a study evaluating wound fluid from 47 patients who had undergone mastectomy, one group found that the 11% of surgical wounds that later developed an infection had significantly lower concentrations platelet derived growth factor (PDGF) and endothelial cell growth factor (ECGF) on post-operative day 1 than the non-infected wounds ($p < 0.05$). They also found that 62% of the patients who developed a seroma had significantly lower levels of basic fibroblast growth factor (bFGF) than those patients who did not develop a seroma ($p < 0.05$) (Baker, Kumar, Melling, Whetter, & Leaper, 2008). Tarlton, Vickery, Leaper, and Baily (1997) found that a decline in MMP-9 levels between 24 and 48 hours post-operatively was a predictor of infection. In another study, Chow, Loo, Yuen and Cheng (2003) found that high levels of IL-4 in wound fluid on post-operative day 1 or high levels of IL-6 on post-operative day 2 in women who had undergone mastectomy was associated with risk of reconstructive flap necrosis. These studies illustrate the utility for examining the process of wound healing using wound fluid in order to advance understanding of the potential mechanisms and modifying factors that ultimately might be targeted for improving outcomes.

Theoretical Framework

An integration of Lazarus and Folkman's cognitive appraisal model of stress and coping and the McCain, Gray, Walter, and Robins (2005) model of the psychoneuroimmunology (PNI) framework guided this study. According to Lazarus and Folkman (1984), stress is a subjective experience that occurs when a person appraises an event to be stressful and demands exceed available resources to cope or deal with that event. This interaction between the environment or stressor and the person who perceives or appraises the event as stressful is referred to as the transactional model of stress. PNI is a theoretical perspective that utilizes the transactional model of stress to explore the complex interactions and relationships between behaviors and the neuroendocrine and immune systems and the environmental and psychosocial factors that may moderate these interactions (Caudell, 1996; McCain, Gray, Walter & Robins, 2005, Zeller, McCain & Swanson, 1996). The PNI model proposed by McCain et al. (2005) provides a framework to evaluate the complex interactions between behavior and its effects on health and wellbeing. "The major purpose of PNI research is to determine whether a valid association exists between risk factors such as stressors, depression or pain and the outcome" (Zeller, McCain, McCann, Swanson, & Colletti, 1996, p. 314). Evidence supports the association between psychological stress and immune system functioning (Segerstrom & Miller, 2004), and cell mediated immunity, including interactions between various biochemical mediators, plays an important role in the early inflammatory stages of wound healing. It is through this pathway that stress may impair wound healing, by attenuating the initial inflammatory phase of the wound healing response and thus disrupting the orderly progression of normal wound healing (Hubner et al., 1996; Lucas, 2011).

Research Objectives

The purpose of this study was to first identify patterns of biochemical mediators present in acute surgical wound fluid over time. Additionally, we wanted to describe the relationship of psychological stress and the biochemical mediators of wound healing in the local wound environment.

Method

Design

This study used a descriptive non-experimental design with samples collected over time to describe biochemical patterns (including selected cytokines, chemokines, and growth factors) in surgical wounds in women undergoing breast reconstruction at a southeastern United States urban academic medical center. Following institutional board review approval, participants were recruited from the plastic and reconstructive surgery office over a 38-month time period.

Participants

The target population was women referred to the Virginia Commonwealth University (VCU) Plastic and Reconstructive Surgery practice for autologous breast reconstruction following mastectomy for a diagnosis of breast cancer. This convenience sample was recruited prospectively as they presented to the practice for their pre-operative visits. This visit followed the consultation visit with the plastic surgeon and the type of reconstruction had been determined prior to the pre-operative research enrollment visit.

From this target population, 20 English-speaking women aged 21-65 with breast cancer or a history of breast cancer and who were scheduled to have abdominally based

autologous tissue reconstruction were enrolled in the study. For consideration of autologous abdominal tissue reconstruction, a multitude of factors are taken into consideration to identify appropriate surgical candidates. The length and complexity of these reconstructive surgical procedures require that patients be evaluated based on risk factors proposed by Hartrampf (1991), which include psychological stability, diabetes, vascular disease, weight, smoking history, past abdominal surgeries, pulmonary disease and cardiac disease. Essentially, women who are considered good surgical candidates and selected by the surgeon for the procedure are in good health except for their cancer diagnosis. Also excluded from the study were patients on immunosuppressive medications, women undergoing hysterectomy and/or oophorectomy at the time of mastectomy, women who were pregnant or women with a medical history of autoimmune disorders. Autologous breast reconstruction provides a good model for studying acute wound healing because the abdominal incision is a clean wound uncomplicated by the biological factors and treatment-related factors associated with breast cancer, such as radiation. The resulting abdominal wound has a large surface area that drains easily with conventional Jackson-Pratt drains.

Procedure

After full discussion of the study, documented informed consent was obtained. Data collected included demographic information, cancer type, dates of surgeries, menstrual cycle data, relevant past surgical history, and chemotherapy and radiation history. Patients usually came to the clinic approximately 7-10 days before their scheduled surgery for a pre-operative history and physical examination. The perceived stress questionnaires were administered at that time. The 10-item Perceived Stress Scale

(PSS) (Cohen, Kamarck, & Mermelstein, 1983), 100mm Visual Analog Scale (VAS) measuring stress, and the 22-item Impact of Events Scale-Revised (IES-R) (Creamer, Bell, & Failla, 2003) were administered to evaluate pre-operative perceived stress levels. Approximately 2 ml of whole blood was collected in concert with other pre-operative blood work in order to evaluate peripheral blood levels of the same biochemical mediators measured in the wound fluid.

Following a participant's reconstructive surgery, wound fluid from the abdominal surgical site was obtained from the tubing of the Jackson-Pratt drain routinely placed in the surgical site during surgery for post-operative serous fluid management. Fluid was collected from the wound at 24, 48, 72, and 96 hours post-operatively, and immediately processed and stored at -70 C in the VCU Center for Biobehavioral Clinical Research (CBCR) until all samples had been collected from all participants. Pain was assessed at each sample collection point, along with type of pain management being instituted. Pain was rated on a scale of 0-10, with 0 being no pain and 10 being the worst pain. At the 48-hour collection, the IES-R and the stress VAS were re-administered and an additional blood sample was collected for peripheral blood analysis of the targeted biochemical mediators at this time.

Following hospital discharge, participants were followed weekly to biweekly as part of their routine post-surgical care. Surgical complications such as the development of seroma, flap necrosis, or infection, were evaluated during this time and were documented if they occurred.

Measurement of Biochemical Mediators

Levels of selected cytokines, chemokines, and growth factors in the wound fluid and plasma were determined using a Bio-Plex Plus® magnetic bead array system (BioRad, Inc.) with the 27-plex standardized detection kit according to the kit manufacturer's protocol. Assays were performed in the VCU School of Nursing Center for Biobehavioral Clinical Research laboratory. The Bio-Plex assay combines fluorescent flow cytometry and ELISA technology, providing simultaneous quantitation of each of the 27 analytes using an analyte quantity as small as 12 µl. The manufacturer reports that the assay accurately measures analyte values in the range of 1-2,500 pg/ml (well within the limits of detection for this project), is precise (intra-assay CV < 10%, inter-assay CV < 15%), and shows less than 1% cross-reactivity among cytokines or with other molecules. The biochemical mediators detected by this standardized 27-plex kit are summarized in Appendix 1.

Questionnaires and Scales

Stress was first measured by administration of the 10-item PSS (Cohen, Kamarck, & Mermelstein, 1983). The PSS was developed based on Lazarus and Folkman's transactional model of stress and was intended to evaluate the degree to which respondents perceived their lives to be unpredictable, uncontrollable, and overloaded (Cohen et al., 1983; Monroe, 2007). This questionnaire consists of 5 negatively formatted and 5 positively formatted questions that address nonspecific appraised stress during the month prior to completing the questionnaire (Cohen et al., 1983). The PSS was administered at the pre-operative visit.

A VAS is designed to measure the intensity, strength, or magnitude of individuals' sensations and subjective feelings and opinions about a specific stimulus

(Waltz, Strickland & Lenz, 2005). For this study, a 100 mm VAS was completed by participants before surgery at their pre-operative visit and at 48 hours post-operatively. Participants were instructed to mark the line after being asked the question, “How much do you feel stressed right now?” The farthest left point is marked 0 or “not at all”, the farthest right point is marked 10 or “extremely.”

The IES-R (Weiss & Marmar, 1997) is a widely used self-report measure for subjective distress related to any traumatic life event. There are 22 items divided into 3 subscales, intrusion, avoidance, and hyperarousal. The scale asks about certain events and their frequency of occurrence over the past week. Participants are asked to rate each item in the IES-R on a scale of 0 (not at all), 1 (a little bit), 2 (moderately), 3 (quite a bit), or 4 (extremely). A total score of 33 or a average score over 1.5 or higher indicates powerful impact or traumatic stress levels (Zhang, Y & Ho, 2011). Scores higher than 44 indicate severe impact and may adversely affect functioning (Meisel et al., 2012). This scale was administered with the stress VAS at baseline and again at 48 hours post-operatively.

Data Analysis

Statistical analysis was performed using JMP software, version 12. All demographic data were summarized using descriptive statistics (i.e., means and variance for continuous data and frequencies for the categorical data). Biological data were \log_{10} transformed for statistical manipulation and statistical analysis was carried out using mixed model analysis to show trends among cytokines over time. Subject was the fixed effect, level of cytokine was the random effect. Using profile plots, the biological data were displayed over time both within and across the biochemical mediators. These plots were visually examined for patterns.

Levels of perceived stress were examined descriptively with the intention of detecting associative trends with the biological data. Correspondence of peripheral blood and wound fluid mediators were determined using the Pearson's correlation. A p -value of ≤ 0.05 was considered to be statistically significant.

Findings

A total of 20 autologous breast reconstruction patients were enrolled in the study between September 30, 2008 and December 15, 2011. The age range participants was 38-63 years with a mean (\pm standard deviation [SD]) of 50.2 (\pm 8.2). The BMI range was 19.3-31.8 with a mean of 26.3 (\pm 3.5). The majority of participants (70%) were white; all others were black. Overall, these patients were healthy with few reported comorbidities, including depression (30%) and hypertension (15%). All participants were post-menopausal, either reporting not having a period in the past year or that they had undergone hysterectomy at some time prior to their reconstruction (70%).

Participants undergoing immediate versus delayed reconstruction were equally divided; the type of reconstruction is summarized in Table 1. Among the 10 participants who underwent delayed reconstruction, 4 had a history of failed tissue expander reconstruction in the past, and 8 had a previous history of radiation. Three of the 10 patients who had immediate reconstruction had a history of radiation following segmental mastectomy for their cancer diagnosis. Cancer type and treatment also are summarized in Table 1.

Table 1

Cancer Type and Previous Treatment

Cancer Type	Timing of Reconstruction	
	Delayed	Immediate
DCIS	1	3
DCIS & IDC	0	1
IDC	5	6
ILC	2	0
Inflammatory	2	0
History of Radiation		
No	2	7
Yes	8	3

Note: Delayed Reconstruction take place after the reconstruction, Immediate reconstruction takes place immediately following the mastectomy. DCIS = ductal carcinoma in situ; IDC = infiltrating ductal carcinoma; ILC = invasive lobular carcinoma

Intraoperative complications occurred in 4 of the 20 participants. Flap failure subsequently occurred in 2 of these 4 participants. Two participants required intraoperative blood transfusions.

In the immediate post-operative phase, 2 participants suffered migraine headaches and one developed post-operative hypertension, which delayed her discharge by 2 days. One participant developed a hematoma and another developed an arterial thrombosis in the reconstructive site; both returned to the operative suite and both reconstructions were successfully salvaged. One participant developed a large pulmonary saddle embolus which required surgical intervention and the implementation of anticoagulants, which caused extensive bleeding from the surgical sites and lead to multiple blood transfusions. Her data set was incomplete. However, her data collected prior to this significant complication was included in the data analysis.

Three of the 20 participants developed post-discharge complications. Two women experienced some distal tip necrosis of the transferred flap, which went on to heal without complications. Another developed cellulitis upon discharge and was readmitted for antibiotic therapy and had no further complications.

Psychometric Measures of Stress

In this study, we found low levels of stress preoperatively. All psychometric stress measures showed a significant positive correlation ($p < 0.05$) and are presented in table 3. Pre-operatively, participants were asked to complete the PSS, IES-R, and the VAS. The mean score for the pre-operative PSS was 19.6, with a median of 20.5 and a standard error of 1.86; scores ranged from 3 to 37. Pre-operative IES-R scores also indicated that stress levels were low at this time point. Mean IES scores were 1.26, with a median of 1.32 and a standard error of 0.18; scores ranged from 0.8 to 3.04. VAS scores indicated a slightly higher stress level, but still not severe. Preoperatively, the mean score on the

VAS was 52.66 with a median score of 48.98, standard error of 7.45, and a range of 2.02 to 100.

Stress scores did not vary much over time. Post-operative stress scales were administered 48 hours post-surgery. Post-operative IES median scores were 1.4, with a median score of 1.27, a standard error of 0.19, and a range from 0.20 to 2.71. Post-operative VAS scores were 47.14 with a median score of 49.25, standard error of 7.99, and a range from 2.04 to 100. Table 2 displays the high intercorrelations among all psychological stress measures.

Table 2

Pearson's Correlations for Stress Measures

Time	Stress Measure	Stress Measure	N	Correlation
24 Hours	IES	PSS	20	0.5985*
	IES	VAS	15	0.6008*
	PSS	VAS	15	0.7152*
48 Hours	IES	VAS	14	0.6945*

Note: IES = Impact of Events Scale; PSS = Perceived Stress Scale; VAS = 100mm Visual Analog Scale

Wound Fluid Biochemical Profiles

All the selected biochemical mediators were detected at some level in the wound fluid, although some percentages of detection were quite low. In particular, eotaxin and IL-15 levels were detected less than 30% of the time. Differential levels of measured wound fluid mediators were observed for each post-operative day and are summarized in Table 3.

Table 3

Wound Fluid Cytokine Concentrations (pg/ml) Over Time

Cytokine	24 Hours Post-Op			48 Hours Post-Op			72 Hours Post-Op			96 Hours Post-Op		
	Median	Min, Max	% Above Detection	Median	Min, Max	% Above Detection	Median	Min, Max	% Above Detection	Median	Min, Max	% Above Detection
IL-1 β	99.10	(10.78, 1027.91)	100	29.06	(4.36, 217.48)	100	13.49	(1.66, 69.40)	100	13.56	(2.39, 47.00)	100
IL-1ra	6625.92	(1055.03, 20000.00)	100	4637.15	(1395.93, 11087.80)	100	4916.57	(209.10, 10024.5)	100	4915.02	(1008.52, 9700.22)	100
IL-2	5.59	(0.10, 62.02)	75	0.10	(0.10, 9.82)	45	0.10	(0.10, 11.55)	17	0.10	(0.10, 8.47)	30
IL-4	2.45	(1.19, 4.90)	100	1.89	(0.40, 8.75)	100	1.58	(0.03, 3.36)	95	1.71	(0.18, 2.95)	100
IL-5	3.63	(1.87, 50.80)	100	7.52	(1.67, 224.60)	100	10.99	(2.35, 222.37)	100	13.23	(7.03, 262.94)	100
IL-6	56489.28	(18587.81, 257225.73)	100	28656.34	(2734.93, 91394.87)	100	9073.22	(1353.13, 144174.28)	100	5260.72	(1956.74, 19953.05)	100
IL-7	20.31	(8.59, 101.23)	100	19.11	(8.29, 102.84)	100	18.47	(6.58, 30.64)	100	21.76	(9.31, 55.30)	100
IL-8	4452.19	(828.01, 14920.91)	100	2556.82	(583.04, 9841.64)	100	1815.20	(174.01, 9910.90)	100	3026.84	(433.97, 20433.57)	100
IL-9	20.45	(0.10, 10728.23)	95	13.95	(0.10, 1760.89)	85	7.59	(0.10, 348.87)	78	7.73	(0.10, 359.11)	50
IL-10	119.56	(34.60, 301.34)	100	75.38	(18.05, 159.53)	100	44.67	(3.84, 95.18)	100	54.04	(15.40, 98.58)	100
IL-12	262.00	(65.73, 454.11)	100	244.32	(80.23, 450.73)	100	223.88	(14.34, 534.52)	100	262.97	(71.99, 503.50)	100
IL-13	88.77	(46.47, 213.65)	100	80.12	(17.09, 149.75)	100	78.65	(31.06, 137.32)	100	75.26	(44.90, 119.94)	100
IL-15	0.10	(0.10, 22.19)	5	0.10	(0.10, 0.10)	0	0.10	(0.10, 0.10)	0	0.10	(0.10, 19.50)	20
IL-17	45.41	(0.10, 114.89)	95	18.42	(0.10, 125.02)	80	11.13	(0.10, 43.28)	72	1.96	(0.10, 40.42)	50

Perceived Stress and Surgical Wound Cytokine Patterns

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Table 3 Continued: Wound Fluid Cytokine Concentrations (pg/ml) Over Time

Eotaxin	0.10	(0.10, 206.96)	20	0.10	(0.10, 76.56)	25	0.10	(0.10, 123.34)	22	0.10	(0.10, 563.34)	40
FGF-B	108.11	(15.43, 258.90)	100	44.47	(0.10, 105.29)	95	32.11	(0.10, 78.80)	83	11.16	(0.10, 59.55)	70
G-CSF	2905.93	(695.92, 9000.00)	100	641.23	(100.15, 7853.37)	100	125.01	(24.19, 1011.87)	100	151.39	(29.75, 398.60)	100
GM-CSF	152.04	(15.99, 1735.42)	100	121.43	(17.08, 576.73)	100	99.97	(15.69, 277.05)	100	95.16	(3.76, 297.60)	100
IFN- γ	184.82	(101.55, 307.76)	100	148.19	(64.90, 294.48)	100	148.42	(15.20, 310.96)	100	165.63	(31.46, 251.36)	100
IP-10	4502.75	(571.16, 36148.43)	100	4409.04	(865.25, 23078.36)	100	5487.88	(462.46, 50000.00)	100	4309.51	(883.89, 50000.00)	100
MCP-1	3278.38	(2102.40, 4575.40)	100	2526.23	(500.60, 3238.87)	100	2286.12	(1310.25, 3597.04)	100	2206.73	(1464.71, 2963.51)	100
MIP-1 α	37.84	(6.36, 315.23)	100	10.25	(4.50, 131.74)	100	12.77	(0.56, 40.93)	100	11.48	(2.24, 200.65)	100
PDGF-bb	266.78	(63.81, 6561.56)	100	169.12	(50.15, 10000.00)	100	154.27	(3.62, 3129.84)	100	126.71	(19.22, 2646.23)	100
MIP-1 β	1538.40	(220.52, 7327.41)	100	419.48	(162.63, 3936.04)	100	300.88	(98.27, 829.54)	100	369.93	(113.31, 1662.61)	100
RANTES	928.28	(30.78, 4683.14)	100	143.74	(4.70, 6845.77)	100	51.30	(2.01, 622.37)	100	36.45	(0.10, 360.12)	100
TNF- α	45.52	(19.05, 171.28)	100	28.64	(7.54, 77.33)	100	21.63	(0.10, 47.02)	95	16.32	(4.58, 113.06)	90
VEGF	1226.91	(259.97, 2547.26)	100	1239.62	(296.12, 2538.70)	100	1067.25	(50.06, 4729.50)	100	1284.91	(238.35, 2921.29)	100

Note: % Above Detection is the percentage of samples for which we can be fairly certain that the compound was present.

Using mixed model analysis to show trends among cytokines over time, IL-1 β , IL-2, IL-6, IL-9, IL-10, IL-17, FGF-basic, G-CSF, MIP-1 α , MIP-1 β , PDGF-bb, RANTES, and TNF- α decreased, and all were significant with the exception of PDGF-bb. IL-5 showed a significant increase over time. IL-8 decreased over 48 to 72 hours, then significantly increased at 96 hours. The remaining concentrations remained stable over time. IL-1ra, IL-6, IL-8, G-CSF, IP-10, MCP1, MIP-1 β , RANTES and VEGF were present in the highest concentrations. Wound fluid concentrations showed trends that were higher on post-operative day 1 than all other time points with the exception of IL-5, which increased over time, and IL-7, IL-12, IL-15, eotaxin, IP-10 and VEGF, which remained stable. Plots illustrating some of the wound fluid mediator changes over time are presented in Appendix B.

Serum Biochemical Mediator Profiles

The same panel of cytokine, chemokine, and growth factor levels was measured in serum preoperatively and 48 hours post-operatively again using mixed model analysis to show trends among cytokines over time; levels are summarized in Table 4. A significant decrease over time was observed in IL-1 β , IL-4, IL-5, IL-7, IL-10, IL-12, IL-13, IL-17, FGF-basic, IFN- γ , IP-10, MIP-1 α , MIP-1 β , PDGF-bb, TNF α , and VEGF. Both IL-6 and MCP-1 showed significant increases over time.

Table 4

Serum Cytokine Concentrations (pg/ml) Over Time and Percentage of Samples Above the Minimal Detection Level

Cytokine	24 Hours Post-Op			48 Hours Post-Op		
	Median Conc.	Min, Max	% Above Detection	Median Conc.	Min, Max	% Above Detection
IL-1 β	4.68	(2.09, 258.32)	100	2.74	(0.94, 5.9)	100
IL-1ra	337.88	(121.52, 9770.27)	100	220.52	(82.94, 1531.51)	100
IL-2	6.00	(0.10, 68.90)	61	1.07	(0.10, 17.74)	50
IL-4	5.98	(1.54, 12.09)	100	5.80	(3.20, 12.14)	100
IL-5	6.01	(1.31, 15.20)	100	4.94	(2.27, 11.71)	100
IL-6	18.20	(7.14, 972455.02)	100	46.17	(13.01, 200.50)	100
IL-7	14.20	(5.82, 25.16)	100	9.57	(4.92, 20.16)	100
IL-8	29.59	(16.61, 6571.63)	100	25.93	(18.36, 55.60)	100
IL-9	20.07	(0.10, 18143.43)	89	5.75	(0.10, 12185.77)	58
IL-10	9.65	(0.10, 166.08)	95	5.01	(0.10, 21.86)	75
IL-12	27.27	(6.77, 352.09)	100	17.75	(0.10, 34.63)	92
IL-13	6.09	(1.93, 106.02)	100	4.31	(0.10, 18.59)	92
IL-15	0.10	(0.10, 58.28)	11	0.10	(0.10, 0.10)	0
IL-17	81.34	(0.10, 184.97)	95	60.30	(2.94, 150.00)	92
Eotaxin	0.10	(0.10, 284.73)	34	0.10	(0.10, 508.92)	83
FGF-B	58.59	(19.95, 173.04)	100	29.31	(0.10, 65.76)	100
G-CSF	124.08	(74.74, 6574.77)	100	82.93	(25.12, 197.15)	8
GM-CSF	0.10	(0.10, 292.95)	33	0.10	(0.10, 253.06)	8
IFN- γ	209.61	(121.58, 313.55)	100	154.58	(78.79, 284.79)	100
IP-10	613.32	(160.54, 8767.74)	100	271.36	(110.70, 711.67)	100
MCP	34.96	(13.40, 3728.91)	100	35.86	(12.75, 125.61)	100
MIP-1 α	7.78	(4.04, 126.46)	100	4.81	(2.19, 9.52)	100
PDGF-bb	1651.73	(116.28, 5462.59)	100	1612.12	(595.05, 3838.94)	100
MIP-1 β	64.38	(33.52, 2392.48)	100	42.24	(32.31, 61.94)	100
RANTES	2991.03	(494.54, 4574.49)	100	2929.11	(1990.15, 4511.44)	100
TNF- α	44.60	(13.38, 77.03)	100	30.49	(10.95, 69.42)	100
VEGF	27.25	(7.68, 1759.98)	100	17.12	(0.10, 38.30)	92

Note: % Above Detection = percentage of samples for which we can be fairly certain that the compound was present.

Correlation of Stress Measures with Serum and Wound Fluid Mediators

This study showed several significant correlations between stress measures and levels of the selected biochemical mediators. Mediator data were log transformed due to

the large range and non-normal distribution of values. In serum, there was a significant negative correlation between IL-5 and PSS and IES-R scores at baseline. MIP-1 α and MIP-1 β showed significant positive correlations with the IES-R. Two days after surgery, only RANTES showed a significant negative correlation with the IES-R. These data are summarized in Table 5. While there appeared to be high correlations for many of the serum mediators and stress measures, many of them were not significant.

Table 5

Pearson's Correlations of Stress Measures with Serum Cytokines

	Measures at Baseline			Measures at 48 Hours	
	PSS n=18	IES n=18	VAS n=13	IES N=11	VAS N=11
IL-1 β	-0.0028	0.4461	-0.0042	-0.1490	0.2082
IL-1ra	-0.0922	0.3857	-0.1385	0.3266	0.5068
IL-2	-0.1879	0.3991	-0.2173	0.4930	0.4276
IL-4	-0.0207	-0.2203	0.2248	-0.2466	0.0722
IL-5	-0.5051*	-0.4713*	0.1175	0.2505	0.5168
IL-6	-0.0470	0.3976	-0.0007	0.2380	0.0072
IL-7	-0.4468	0.0821	0.1395	-0.1294	0.2429
IL-8	-0.1202	0.3414	0.1512	0.0003	0.0439
IL-9	-0.2366	0.2019	-0.0673	0.1092	0.1700
IL-10	-0.1701	0.3756	0.0089	0.2283	0.5522
IL-12	-0.2043	0.2752	0.3115	0.2456	0.4217
IL-13	-0.0772	0.3683	0.1262	0.3114	0.4152
IL-15	0.3598	0.3671	-0.0682	-0.0000	-0.0000
IL-17	0.4410	0.2607	0.2925	-0.4125	-0.1104
Eotaxin	0.0777	0.0653	0.0497	0.5836	0.4179
FGF-B	-0.1991	0.1428	0.0345	-0.4142	-0.1974
G-CSF	-0.1381	0.3089	-0.2260	-0.4819	-0.2609
GM-CSF	-0.1419	0.4675	0.2890	0.0116	0.0147
IFN- γ	-0.0685	0.3921	0.2849	-0.0327	0.2077
IP-10	0.0661	0.3256	0.0983	-0.1199	0.1019
MCP-1 (MCAF)	-0.0991	0.3107	-0.2268	0.4793	0.1690
MIP-1 α	0.2911	0.5407*	0.2058	-0.4247	0.0263
PDGF-bb	-0.0088	-0.2025	0.2931	0.0259	0.1510
MIP-1 β	0.1102	0.4701*	0.1765	-0.2932	-0.2289
RANTES	0.0385	-0.3253	-0.3141	-0.6104*	-0.5480
TNF- α	-0.1592	0.0564	0.0610	-0.0939	0.2767
VEGF	-0.1882	0.3322	0.3588	0.1569	0.2884

Note. (* p<0.05); IES = Impact of events scale; PSS = Perceived Stress Scale; Analog Scale = 100mm visual analog scale.

Relationships between wound fluid biochemical mediators and stress measures at 48 hours post-surgery were examined. As seen in Table 6, there were some high correlations, but many were not statistically significant. However, there was a significant positive correlation found between IL-2 and IES-R scores and a significant negative correlation between eotaxin and IES-R scores. Significant positive correlations were found between VAS scores and eotaxin, and significant negative correlations were identified between the VAS and IL-12 as well as VEGF.

Table 6

*Pearson's Correlations of Stress Measures with
Wound Fluid Mediators at 48 Hours Post Surgery*

	IES N=14	VAS N=14
IL-1 β	0.4762	0.0218
IL-1ra	0.3055	0.0574
IL-2	0.5839*	0.2653
IL-4	0.1923	0.1895
IL-5	0.3586	0.2754
IL-6	0.0455	-0.4053
IL-7	0.0138	-0.4420
IL-8	0.1439	0.0967
IL-9	0.2638	-0.0778
IL-10	-0.0253	-0.1929
IL-12	-0.2410	-0.5459*
IL-13	0.3101	0.1629
IL-15	0.0000	0.0000
IL-17	0.1496	0.0499
Eotaxin	0.7983*	0.7034*
FGF-B	0.1844	-0.2083
G-CSF	0.2654	-0.1082
GM-CSF	-0.2116	-0.2783
IFN- γ	0.0342	-0.1336
IP-10	0.0040	0.0354
MCP-1	-0.1039	0.3428
MIP-1 α	0.0195	-0.0442
PDGF-bb	0.1968	0.3515
MIP-1 β	0.0582	-0.1293
RANTES	0.0290	0.2936
TNF- α	0.1334	-0.2566
VEGF	0.2945	-0.6063*

Note. (* p<0.05); IES = Impact of events scale; VAS = 100mm visual analog scale.

Discussion

Numerous investigators have shown a relationship between psychological stress and wound healing, and some have specifically explored that association with wound biochemical mediators such as cytokines (Broadbent, Petrie, Alley, & Booth, 2003; Glaser et al., 1999; Kiecolt-Glaser et al., 2005). To our knowledge, this is the first study to explore those relationships in respect to the local surgical wound environment, specifically surgical wound fluid mediators over time. We believe this is also the first study characterizing wound fluids in abdominal muscle flap donor site wounds, a wound type that has no associated pathologic abnormality such as cancer.

We assumed this would be a stressful period for women and that higher stress might affect wound biochemical mediators. However, the low levels of perceived stress, based on the psychological scales used, leads us to believe that our participants were not experiencing high levels of stress and that stress may not have been the most appropriate variable to measure. Additionally, low mean stress scores may have been related to the small sample size, as many of the study participants had high scores on their questionnaires. Perhaps pre-surgical “worry” would have been a better variable to measure. Broadbent et al. (2003) noted that higher levels of pre-surgical worry were associated with greater post-operative pain and longer recovery times, as well as poorer self-ratings on recovery.

Wound healing occurs in complex overlapping phases. In our study, time points fell within the inflammatory phase, so we expected to see higher levels of those cytokines, chemokines, and/or growth factors that are active in the inflammatory phase of healing. Interestingly, we were able to detect all 27 selected mediators at some level in

wound fluid samples and the concentration of all cytokines was greatest on day 1 post-operatively. These findings are similar to those of DiVita et al. (2006) with the exception of VEGF, where they reported higher levels of VEGF on day 4.

We chose to report even those mediator levels that were lower than the 50% detection limit, because even if those concentrations were low, some participants did have detectable levels and thus the data may be meaningful. Some of these cytokines have not been extensively reported for wound fluid, and at this time we do not know what role they may play, if any, in the wound healing cascade. Table 4 provides quantitative measurements of all 27 factors detected in the surgical wound fluid using the Bio-Rad 27-plex plate. Overall, the ranges detected were similar to the concentrations of acute wound fluid cytokines previously reported (Baker & Leaper, 2000). Interestingly, some studies showed varying concentrations of cytokines at the same time point post-operatively. This is most likely due to different assays having different sensitivities and specificities. Differences in surgeries and surgical site may also play a role. For example, Baker and Leaper (2000) demonstrated that cytokine concentrations vary based on the breast surgical site verses the colon surgical site.

Inflammatory cytokine production was highest in the first 24 hours post wounding, which is consistent with other studies (Baker, El-Gaddal, Aitken, & Leaper, 2003; Chow, Loo, Yuen, & Cheng, 2003; DiVita et al., 2006). As reflected in the daily wound fluid profiles, IL-1 β , IL-6, and IL-8 concentrations were greatest at 24 hours and then decreased over time. These mediators are released by neutrophils and monocytes, which are the predominate cell types found in the site of injury in the early inflammatory phase, recruited to the wound by chemotactic factors released during hemostasis and by

mast cells (Li, Chen, & Kirsner, 2007). TNF- α was also found to peak earlier and to decrease over time, a finding consistent with others (Hubner et al., 1996).

IL-6 was present in the highest median concentration (56489.28 pg/ml) of all cytokines on day 1 or any other single time point. The concentration remained high but significantly decreased over time. IL-6 has been identified as an early marker of the systemic inflammatory response and tissue damage (Loo et al., 2007), and thus would be expected to be elevated following a large abdominal surgical operation such as the one in our study. Prolonged elevations in levels of IL-6 have been associated with increased scarring in the wound site (Rumalla & Borah, 2001), but we did not follow our participants long enough to evaluate scarring.

IL-1 β levels declined significantly from 24 to 72 hours post surgery. IL-1 exists in two isoforms, IL-1 α and IL-1 β , and plays a vital role in collagen synthesis, fibroblast growth, and keratinocyte growth, as well as activation of neutrophils (Efron & Moldawer, 2004). However, elevated levels of IL-1 beyond the first 7 days of wound healing have been associated with poor wound healing outcomes (Barone et al., 1998; Trengove, Bielefeldt-Ohmann, & Stacey, 2000). This coincides with our findings in that there were no wound healing complications observed postoperatively.

FGF basic demonstrated a peak at post-operative day 1 and a steadily declined through day 4, which is consistent with previous studies (Baker, Kumar, Melling, & Whetter, 2008; Nissen, Polverini, Gamelli & DiPietro, 1996). FGF basic is thought to initiate angiogenesis in the wound until VEGF can take over (Baker, Kumar, Melling & Whetter, 2008), but both cytokines act synergistically to stimulate endothelial cell function, migration, and proliferation (Barrientos, Stojadinovic, Golinko, Brem, &

Tomic-Canic, 2008). VEGF in our study remained stable from 24 to 96 hours postoperatively. It has been reported that VEGF peaks on day 7 following injury (Kapoor, Howard, Hall, & Appeltoorn, 2004), which was outside of the time frame for our study and thus may explain why we did not observe a significant increase.

Forsberg et al. (2008) found that IL-13 and RANTES levels were suppressed in closed traumatic leg wounds and showed a high correlation with wound dehiscence following closure of those wounds. RANTES and IL-13 were not suppressed in this study. IL-13 remained stable over time and RANTES was found at high levels at 24 hours and then significantly decreased over time. Again, this finding coincides with the fact that our participants had no immediate wound healing problems; our wounds also were surgically precise as opposed to traumatic injuries.

Several significant correlations between stress measures and mediator levels were observed. However, these correlations are not similar to those previously reported in the literature. Part of this may be related to the design of this study. We examined correlations of stress measures with wound fluid over time, while other studies looked at various psychological measures and wound fluid at specific time points. Glaser et al. (1999) examined the relationship of perceived life stress and the production of cytokines in the wound fluid of experimentally created skin blisters. Greater stress was associated with decreased cytokine production (IL-1 α and IL-8) at the blister site 5 hours and 24 hours after wounding. Using the same wound model, Kiecolt-Glaser et al. (2007) looked at the effect of hostile marital relationships on wound fluid cytokines. They found that wound fluid mediators (specifically IL-6, TNF α , and IL-1 β) were decreased after conflict. Broadbent et al. (2003) reported that greater perceived stress 1 month before

surgery led to lower levels of IL-1 in wound fluid 20 hours post surgical procedure.

Again, this inconsistency in our findings is very likely due to the low levels of reported stress in our sample.

Limitations

This pilot study was limited by the small sample size, making statistical inferences difficult. Even so, meaningful biochemical mediator patterns were identified in our sample. Earlier studies by Takamiya, Fujita, Saigusa, and Aoki (2007) also demonstrated that meaningful cytokine patterns can be found with a sample as small as 5.

Because participants had lower-than-anticipated levels of perceived stress, power for analyses of psychological stress and biochemical mediators was limited. All correlations between stress measures and biological measures in the study must be viewed with caution.

Conclusion

The purpose of this study was to gain a better understanding of psychological stress and its potential effects on the surgical wound environment. Stress has been shown to impact health, and wound healing is an additional area in which increased psychological stress has been associated with poorer outcomes. Identifying those individuals who may be experiencing stress and implementing interventions to lower that stress may have a positive impact on their health, and specifically on wound healing. While participants in our study overall did not show high levels of stress, characterization of the surgical wound environment and its biochemical mediators over time provides valuable insight into the physiological mechanisms of wound healing.

Wound fluid characterization provides the opportunity to obtain information reflecting the status of the wound at specific time points and holds potential for the development of specific biomarkers of impaired healing (Yager, 2007). Generally, “biomarkers” are biochemical mediators clinicians use to guide clinical practice. Cardiology, immunology, and endocrinology all use biomarkers in practice, yet traditional serum biomarkers may not be the most appropriate measures to assist in decision making in the case of wound healing. Biomarkers in wound fluid are produced locally in the wound bed, are the most characteristic of activity in the wound environment, and are less susceptible to the influences of systemic elevation of white blood cells and pro-inflammatory cytokines following trauma, treatment interventions such as surgery, or acute elevations in circulating pro-inflammatory cytokines secondary to infection or trauma (Forsberg et al., 2008). Thus, wound fluid biomarkers may ultimately prove to be clinically valuable for assessing wound healing.

Wound fluid biomarkers may provide insight into specific mediators that, when deficient or present in excess, contribute to poor or delayed healing and may one day guide specific treatment options for wounds. Such knowledge would guide decisions to either replace the deficient factor or provide an agonist or one that may down-regulate other inflammatory mediators. Wound fluids may one day serve as prognostic markers of healing, may aid in predicting adverse wound outcomes, and may be beneficial in planning additional therapies post-surgery such as radiotherapy or chemotherapy. We may be able evaluate wound fluids in order to optimize outcomes and prevent complications such as slowed wound healing, infection, seroma development, and tissue flap necrosis.

Understanding the role of specific cytokines in wound healing may lead to the development of direct wound healing therapies to hasten healing in the those patients suffering from chronic wounds. One current example is that of commercially prepared recombinant human variants of PDGF-bb (Becaplermin) that have been shown to clinically improve angiogenesis, collagen deposition, and granulation tissue formation in diabetic neuropathic foot ulcers and pressure ulcers.

Lastly, this study paves the way for additional work in PNI and wound healing. While overall our population was not stressed, there were some correlations among the wound fluid environment and stress. Further research involving the analysis of wound fluid may provide insight into the associations of psychological stress with cellular mechanisms in the local wound site.

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APPENDIX A

27-Plex Chemical Mediators, Their Source and Their Roles

Mediator	Source	Wound Healing Function	Systemic Function
Interleukin 1 β (IL-1 β)	- Produced by neutrophils, monocytes, macrophages and keratinocytes. - Keratinocytes release IL-1 immediately upon wounding (4,5)	- Stimulates keratinocyte migration and proliferation as well as activates fibroblasts and stimulates secretion of FGF-7 (4,5)	- Mediates the action of macrophages, B and T cells, inflammation and fever, acute phase response, hematopoiesis
Interleukin 1 α (IL-1 α)	- Variety of immune cells, adipocytes and epithelial cells	- Natural antagonist of IL-1 function	- Natural antagonist of IL-1 function
Interleukin 2 (IL-2)	- T lymphocytes, NK cells (1)	- T-cell activation - Involved in infiltration of inflammatory cells and fibroblasts (1)	- Augments proliferation of T-lymphocytes, B-cell proliferation and NK cell activity (17)
Interleukin 4 (IL-4)	- Basophils, Helper T-Cells, Mast cells (11)	- Stimulates Fibroblast proliferation - Involved in collagen synthesis - Inhibits TNF α , IL-1 and IL-6 expression (3)	- Inhibits production of inflammatory cytokines (18)
Interleukin 5 (IL-5)	- Helper T cells, Mast cells (11)	- Promote the infiltration of eosinophils into the wound bed (21)	- Eosinophil growth and differentiation (11, 21)
Interleukin 6 (IL-6)	- Polymorphonuclear cells (PMNs) and fibroblasts 2 - Vascular endothelial cells - Activated T Cells (11)	- Potent stimulator of fibroblast proliferation - Inflammation, angiogenesis, re-epithelialization, collagen deposition, tissue remodeling (24)	- Promotes inflammation - Induces acute phase proteins - Activates helper T (Th) cells, - Promotes antibody synthesis - Promotes hematopoiesis (11)
Interleukin 7 (IL-7)	- Stromal cells of bone marrow and thymus and keratinocytes (16)	- Immune response - Involved in extracellular matrix production (16)	- Stem cell differentiation - Promotes immune effector function in T-lymphocytes, NK-cells, monocytes and macrophages (16)
Interleukin 8 (IL-8)	- Macrophages - Endothelial cells - Lymphocytes (11)	- Macrophage and PMN activation and chemotaxis - Involved in keratinocyte maturation (3)	- Acts as a neutrophil chemoattractant (11)
Interleukin 9 (IL-9)	- Activated helper T-lymphocytes (11)	- Function in wound healing has not been identified	- Stimulates T-lymphocytes, and mast cells, epithelial cells, macrophages, eosinophils. - Decreases inflammatory effects of monocytes and macrophages (25)
Interleukin 10 (IL-10)	- Released by macrophages and T lymphocytes (1)	- Inhibits gene expression and synthesis of those cytokines that are thought to be inflammatory, such as TNF- α , IL-1 and IL-6. - Inhibits macrophage and PMN activation (1)	- Cytokine production - Inhibits Th1 cell activity (11)

Mediator	Source	Wound Healing Function	Systemic Function
Interleukin 12 (IL-12)	- T-cells - macrophages - Dendritic cells (11)	-Function in wound healing has not been identified	- Activates NK Cells - T-cell activation (11)
Interleukin 13 (IL-13)	- Activated T cells (11)	- Inhibits macrophage activity (23)	- Activates B-cells - Inhibits Th1 cells - Inhibits proinflammatory cytokine production - Enhances IFN- γ production (11)
Interleukin 15 (IL-15)	- Monocytes (11)	- May play a role in proliferation of regulatory T-cells involved in inflammation (28)	- Stimulates growth of T and NK cells (11)
Interleukin 17 (IL-17)	- Activated memory CD4+ cells (11)	- Low levels may play a role in chronic infections of nails, skin and mucosa by <i>Candida albicans</i> (29)	- Induce synthesis of cytokines that promote T-cell dependent inflammation (11)
Eotaxin	- Produced by a variety of tissues and cells types (13)	- Eosinophil and endothelial cell chemotaxis (13,15)	-Eosinophil chemotaxis (13)
FGF-basic	-Released by macrophages and endothelial cells (1,3)	- Important mediator of wound angiogenesis and epithelialization. - Stimulates fibroblast and keratinocyte proliferation and migration (1,3)	Response to injury, divers role in cell regulation, proliferation, migration and differentiation during embryonic development (15)
Granulocyte colony-stimulating factor (G-CSF)	- A variety of cells including bone marrow stromal cells, fibroblasts, endothelial cells and macrophages (19)	- Modulates immune response - Promotes keratinocyte proliferation	-Enhances myeloid cell proliferation, maturation and function (19)
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	- T-cells - Fibroblasts	- Enhances neutrophil number and function at the wound site. - Increases keratinocyte proliferation - Up regulates IL-6 (4)	- Stimulates proliferation, differentiation and enhances function of neutrophils, monocytes, macrophages, eosinophils and dendritic cells (27)
Interferon- γ (IFN- γ)	- Lymphocytes and NK Cells (12, 20)	- Inhibit fibroblast chemotaxis and proliferation - Impacts collagen production (12)	-Variety of immunological functions - Activates macrophages (20)
Interferon- γ -inducible protein-10 (IP-10)	- T-cells and NK cells (26)	- Associated with lymphocyte production (14)	- Inhibits endothelial cell proliferation - Anti-angiogenic (26)
Monocyte chemo attractant protein-1 (MCP-1)(MCAF)	-Keratinocytes, endothelial cells, macrophages (14)	- Chemo attractant and activator of monocytes, mast cells and lymphocytes (14)	- Stimulates monocytes, memory T cells and NK cells (10)
Macrophage inflammatory protein (MIP-1 α)	- Lymphocytes (11)	- Chemo attractant for monocytes and T cells (10)	- Chemo attractant for monocytes and T cells - Antiviral defense (10)
Macrophage inflammatory protein (MIP-1 β)	- Fibroblasts (11)	- Chemo attractant for monocytes and T cells (10)	- Chemo attractant for monocytes and T cells (10)

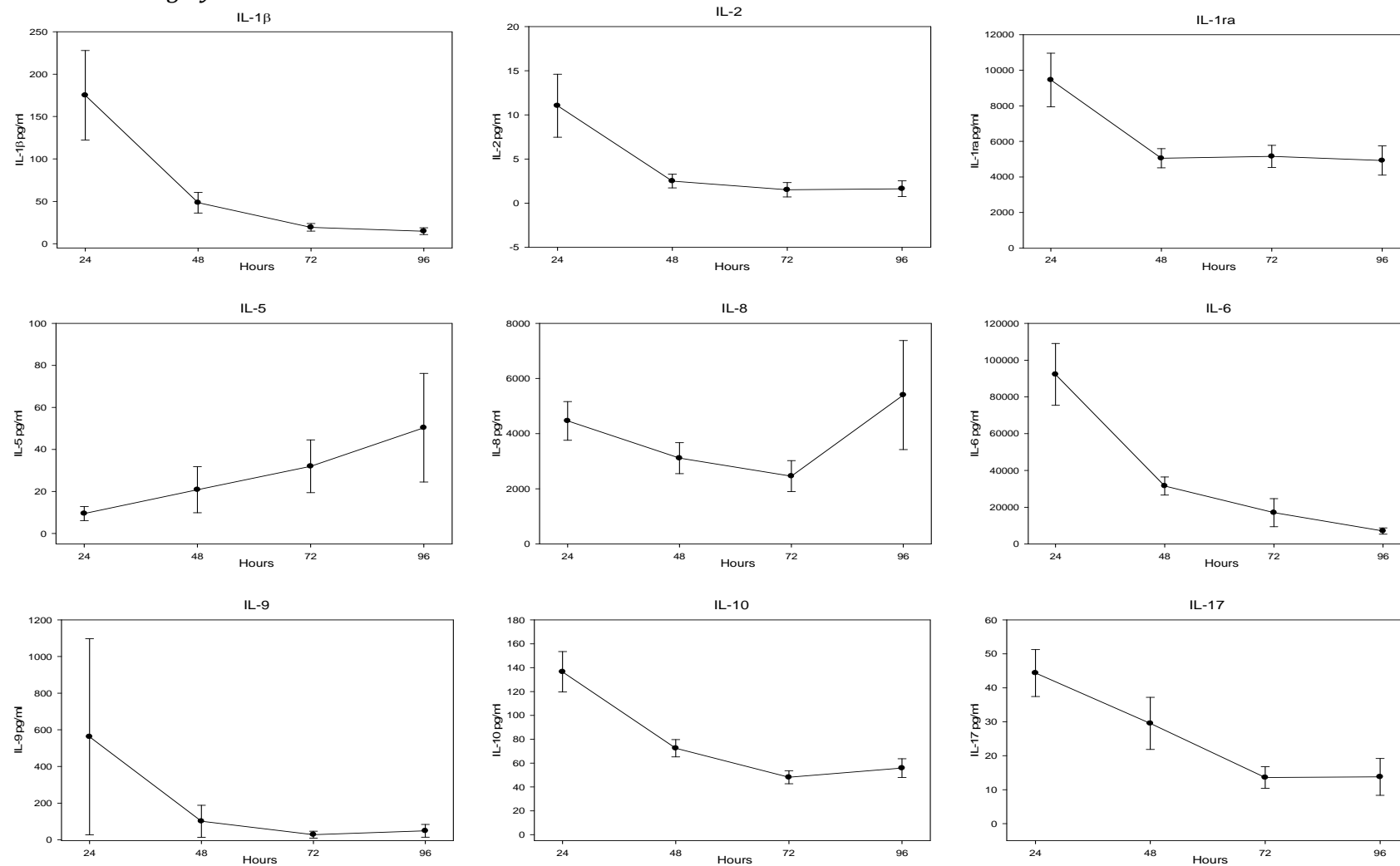
Mediator	Source	Wound Healing Function	Systemic Function
<p>Platelet Derived Growth Factor (PDGF-bb)</p> <p>(PDGF-bb is one of three isomers of PDGF, including PDGF-aa and PDGF-ab, and is the most widely studied.)</p>	- Synthesized by many different cell types, but it is usually the platelets, macrophages and keratinocytes which are responsible for synthesis of this growth factor (1)	<p>- Potent mitogenic, chemotactic and angiogenic growth factor essential in initiating and sustaining wound healing (1,9)</p> <p>- Chemo attractant for neutrophils, monocytes.</p> <p>- Stimulates activation of macrophages</p> <p>- Essential in collagen synthesis and collagenase activation (12)</p>	- Activation of immune cells and fibroblasts (1)
Regulated upon activation normal T-cell expressed and secreted (RANTES)	-Produced mainly by keratinocytes during tissue repair (6, 7)	<p>- Attracts eosinophils, T lymphocytes and monocytes</p> <p>- Involved in angiogenesis (6,7)</p>	- Monocyte/macrophage T-cell chemotaxis (6,7)
Tumor Necrosis Factor (TNF- α)	<p>-Released primarily by macrophages</p> <p>- T-cells</p> <p>- B-cells</p> <p>- NK cells (11)</p>	<p>-Crucial in initiating immune system cascade at the time of injury</p> <p>-Recruitment and maturation of the cellular components of inflammation.(8, 11)</p> <p>-Involved in hemostasis, increased vascular permeability and increased vascular proliferation (1)</p>	<p>- Endothelial activation, cytokine expression in macrophages.</p> <p>- Initiates immune cascade during host response to injury or bacteria</p> <p>- Promotes inflammation (11)</p>
Vascular Endothelial Growth Factor (VEGF)	Platelets, neutrophils, macrophages, endothelial cells, smooth muscle cells, keratinocytes, fibroblasts (24)	- Angiogenesis and granulation tissue formation (12)	- Angiogenesis (1)

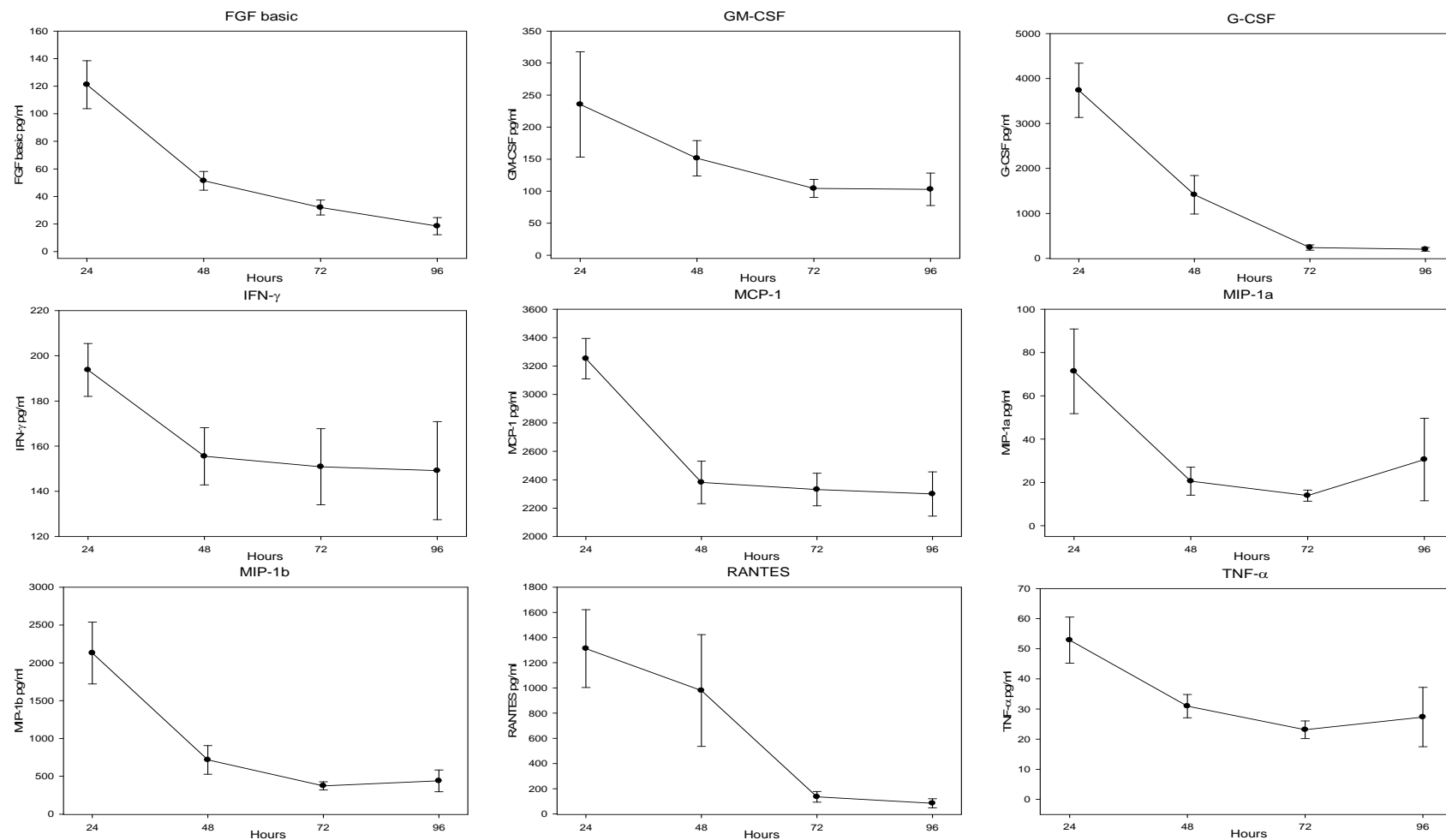
References:

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25. Renauld & Van Snick, 2003
26. Feldman & Libutti, 2003
27. Enzler & Dranoff, 2003
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29. Puel, Cypowyj, Marodi, Abel, Picard & Casanova, 2012

Appendix B

Wound Healing Cytokine Patterns Over Time



Appendix B: Representation of some of the daily changes of cytokine concentrations in drain fluid (DF). DF was collected at 24, 48, 72 and 96 hours post-surgery.

DATE: May 12, 2010

TO: Nancy L. McCain, DSN, RN, FAAN
Department of Adult Health Nursing
Box 980567

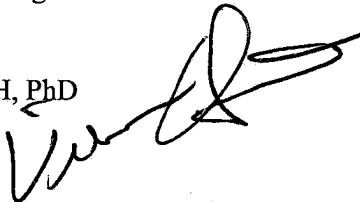
FROM: William E. Smith, PharmD, MPH, PhD
Chairperson, VCU IRB Panel A
Box 980568

RE: **VCU IRB #: HM12936**
Title: A Pilot Study of Perceived Stress and Surgical Wounds Cytokine Patterns

Office of Research
Office of Research Subjects' Protection

Biotech Research Park, Bldg. 1
800 East Leigh Street, Ste. 114
P.O. Box 980568
Richmond, VA 23298-0568

(804) 828-0868 - Phone
(804) 828-1448 - Fax
(804) 828-1120 - TDD



On May 5, 2010, the following research study was approved by expedited review according to 45 CFR 46.110 Category 2, 3 and 7. This approval includes the following items reviewed by this Panel:

RESEARCH APPLICATION/PROPOSAL: None

PROTOCOL: A Pilot Study of Perceived Stress and Surgical Wounds Cytokine Patterns (VCU Research Plan; dated 3/25/10)

CONSENT/ASSENT:

- Research Subject Information and Consent Form (Version 1; dated 3/25/10; 5 pages)

ADDITIONAL DOCUMENTS: None

This approval expires on April 30, 2011. Federal Regulations/VCU Policy and Procedures require continuing review prior to continuation of approval past that date. Continuing Review report forms will be mailed to you prior to the scheduled review.

The Primary Reviewer assigned to your research study is Lyons Hardy, BSN, MSN. If you have any questions, please contact Ms. Hardy at lhardy@mcvh-vcu.edu or 828-2609; or you may contact Stephan Hicks, IRB Coordinator, VCU Office of Research Subjects Protection, at hickssa2@vcu.edu or 828-9876.

Attachment – Conditions of Approval

Conditions of Approval:

In order to comply with federal regulations, industry standards, and the terms of this approval, the investigator must (*as applicable*):

1. Conduct the research as described in and required by the Protocol.
2. Obtain informed consent from all subjects without coercion or undue influence, and provide the potential subject sufficient opportunity to consider whether or not to participate (unless Waiver of Consent is specifically approved or research is exempt).
3. Document informed consent using only the most recently dated consent form bearing the VCU IRB "APPROVED" stamp (unless Waiver of Consent is specifically approved).
4. Provide non-English speaking patients with a translation of the approved Consent Form in the research participant's first language. The Panel must approve the translated version.
5. Obtain prior approval from VCU IRB before implementing any changes whatsoever in the approved protocol or consent form, unless such changes are necessary to protect the safety of human research participants (e.g., permanent/temporary change of PI, addition of performance/collaborative sites, request to include newly incarcerated participants or participants that are wards of the state, addition/deletion of participant groups, etc.). Any departure from these approved documents must be reported to the VCU IRB immediately as an Unanticipated Problem (see #7).
6. Monitor all problems (anticipated and unanticipated) associated with risk to research participants or others.
7. Report Unanticipated Problems (UPs), including protocol deviations, following the VCU IRB requirements and timelines detailed in VCU IRB WPP VIII-7):
8. Obtain prior approval from the VCU IRB before use of any advertisement or other material for recruitment of research participants.
9. Promptly report and/or respond to all inquiries by the VCU IRB concerning the conduct of the approved research when so requested.
10. All protocols that administer acute medical treatment to human research participants must have an emergency preparedness plan. Please refer to VCU guidance on <http://www.research.vcu.edu/irb/guidance.htm>.
11. The VCU IRBs operate under the regulatory authorities as described within:
 - a) U.S. Department of Health and Human Services Title 45 CFR 46, Subparts A, B, C, and D (for all research, regardless of source of funding) and related guidance documents.
 - b) U.S. Food and Drug Administration Chapter I of Title 21 CFR 50 and 56 (for FDA regulated research only) and related guidance documents.
 - c) Commonwealth of Virginia Code of Virginia 32.1 Chapter 5.1 Human Research (for all research).

RESEARCH SUBJECT INFORMATION AND CONSENT FORM



TITLE: A Pilot Study of Perceived Stress and Surgical Wound Cytokine Patterns

VCU IRB PROTOCOL NUMBER:

INVESTIGATORS: Nancy McCain, DSN, RN, FAAN, Valentina S. Lucas, MS, RN, ANP-BC and Andrea Pozez, MD.

This consent form may contain words that you do not understand. Please ask the study doctor or staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

PURPOSE OF THE STUDY

The purpose of this pilot study is to look at how stress may have an effect on how wounds heal. You are being approached about this study because you have been diagnosed with breast cancer and are scheduled for surgery to reconstruct your breast. It has been shown that women who have been diagnosed with breast cancer experience various levels of stress, and that high levels of stress can affect how you heal. Thinking about your surgery can also be stressful. We want to see how that stress might affect the way your surgical incisions heal.

DESCRIPTION OF THE STUDY

If you decide to be in this research study, you will be asked to sign this consent form after all your questions have been answered.

Your participation in this study will last approximately 3 weeks, based on the time of your preoperative appointment and when your surgical drains are removed.

PROCEDURES

If you consent to participate in this pilot study, the following procedures will be performed:

- 1) At your normally scheduled preoperative visit, you will be given two questionnaires to evaluate your level of stress associated with your upcoming surgery. The Perceived Stress Scale consists of 10 questions and the Impact of Events questionnaire consists of 22 questions. You will also be asked to rate your stress on a scale of 0 to 10, 0 being not stress to 10 being severely stressed. You will be given complete privacy and ample time to answer these questions during your preoperative visit.

- 2) Additionally, at this pre-operative visit, you will have 1 additional tube of blood taken when you have your routine blood work done. This additional tube of blood will be about 1 teaspoon.
- 3) Following your surgery, we will take some of the fluid out of your surgical drain to analyze in the laboratory. We will remove the fluid at 24, 48, 72, and 96 hours after your surgery. This fluid is normally measured and thrown away. No extra drains are inserted for this study. The drains that are in place are the normal drains used in breast reconstruction surgery. The drains will not be kept in place longer than what is necessary, and your surgeon, not the study investigator, will make the decision to remove the drain based on your progress after surgery.
- 4) Approximately 48 hours after your surgery, at the same time we collect wound fluid, you will be asked to complete an additional questionnaire. If you are too tired or sedated to read this, then the investigator will read it to you and record your responses. You will also be asked to again rate your stress the 0-10 stress scale.
- 5) Additionally, at this 48-hour time point, another sample of your blood will be taken. We will take 5 cc (about 1 teaspoon) of blood for additional analysis.
- 6) We will also collect some information about you throughout the course of the study including age, cancer diagnosis date, surgery date, health history, last menstrual cycle, length of surgery, pain rating (at each sample collection) and medication used for pain.

RISKS AND DISCOMFORTS

The risk and discomfort associated with the study are, for the most part, no more than those that would be normally associated with undergoing breast reconstruction. The determination of how long your drains will be kept in place is a clinical decision that your surgeon, not the investigator, will make. There may be some discomfort and bruising related to the second blood draw.

BENEFITS TO YOU AND OTHERS

This is not a treatment study, and you are not expected to receive any direct medical benefits from your participation in the study. The information from this research study may lead to a better treatment in the future for people who have surgical wounds or other types of wounds.

COSTS

There will not be any cost for you associated with this study.

PAYMENT FOR PARTICIPATION

You will not be paid for participation in this study.

ALTERNATIVE TREATMENT

Your alternative is not to participate in this study.

CONFIDENTIALITY

Potentially identifiable information about you will be collected in this study and will consist of information from the medical record. Data are being collected only for research purposes. Your data will be identified by a special code number, not your name, and stored separately from medical records in a locked research area. All personal identifying information will be kept in locked or password-protected files. Access to study data will be limited to study personnel only.

You should know that research data may be reviewed or copied by the sponsor of the research or by Virginia Commonwealth University. Personal information about you might be shared with or copied by authorized officials of the Federal Food and Drug Administration, or the Department of Health and Human Services.

Although results of this research may be presented at meetings or in publications, identifiable personal information pertaining to participants will not be disclosed.

COMPENSATION FOR INJURY

Virginia Commonwealth University and the VCU Health System (formerly known as Medical College of Virginia Hospitals) have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study.

If you are injured or if you become ill as a result of your participation in this study, contact your study doctor immediately. Your study doctor will arrange for short-term emergency care or referral if it is needed.

Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Your participation in this study is voluntary. You may decide to not participate in this study. Your decision not to take part will involve no penalty or loss of benefits to which you are otherwise entitled. If you do participate, you may freely withdraw from the study at any time. Your decision to withdraw will involve no penalty or loss of benefits to which you are otherwise entitled.

Your participation in this study may be stopped at any time by the study doctor without your consent. The reasons might include:

- the study doctor thinks it necessary for your health or safety;
- you have not followed study instructions; or
- administrative reasons require your withdrawal.

If you leave the study before its completion no further instructions from us will be necessary. Leaving the study will not affect the care you receive from your surgeon.

QUESTIONS

In the future, you may have questions about your study participation. You may also have questions about a possible side effect or a possible research-related injury. If you have any questions, complaints, or concerns about the research, contact:

Nancy McCain, RN, DSN, FAAN
VCU School of Nursing
804-828-3444

Valentina S. Lucas, RN, MS, ANP-BC
VCU Division of Plastic and Reconstructive Surgery
804-828-3049

If you have questions about your rights as a research subject, you may contact:

Office of Research
Virginia Commonwealth University
800 East Leigh Street, Suite 113
PO Box 980568
Richmond, VA 23298
(804) 827-2157

You may also contact this number for general questions, concerns or complaints about the research. Please call this number if you cannot reach the research team or wish to talk to someone else.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions. Additional information about participation in research studies can be found at <http://www.research.vcu.edu/irb/volunteers.htm>.

CONSENT

I have been provided with an opportunity to read this consent form carefully. All of the questions that I wish to raise concerning this study have been answered.

By signing this consent form, I have not waived any of the legal rights or benefits to which I otherwise would be entitled. My signature indicates that I freely consent to participate in this research study. I will receive a copy of the consent form once I have agreed to participate.

Subject Name, printed

Subject Signature

Date

Name of Person Conducting Informed Consent
Discussion / Witness
(Printed)

Signature of Person Conducting Informed Consent
Discussion / Witness

Date

Investigator Signature (if different from above)

Date

A Pilot Study of Perceived Stress and Surgical Wound Cytokine Patterns

IMPACT OF EVENT SCALE-REVISED

Weiss, D. S., & Marmar, C. R. (1997). The Impact of Event Scale—Revised. In J. P. Wilson, & T. M. Keane (Eds.), *Assessing psychological trauma and PTSD: A handbook for practitioners* (pp. 399–411). New York: Guilford Press.

Instructions: The following is a list of difficulties people sometimes have after stressful life events. Please read each item, and then indicate how distressing each difficulty has been for you *during the past 7 days* with respect to your surgery. How much were you distressed or bothered by these difficulties?

		Not at all	A little bit	Moder- ately	Quite a bit	Extreme- ly
1	Any reminder brought back feelings about it.	0	1	2	3	4
2	I had trouble staying asleep.	0	1	2	3	4
3	Other things kept making me think about it.	0	1	2	3	4
4	I felt irritable and angry.	0	1	2	3	4
5	I avoided letting myself get upset when I thought about it or was reminded of it.	0	1	2	3	4
6	I thought about it when I didn't mean to.	0	1	2	3	4
7	I felt as if it hadn't happened or wasn't real.	0	1	2	3	4
8	I stayed away from reminders about it.	0	1	2	3	4
9	Pictures about it popped into my mind.	0	1	2	3	4
10	I was jumpy and easily startled.	0	1	2	3	4
11	I tried not to think about it.	0	1	2	3	4
12	I was aware that I still had a lot of feelings about it, but I didn't deal with them.	0	1	2	3	4
13	My feelings about it were kind of numb.	0	1	2	3	4
14	I found myself acting or feeling like I was back at that time.	0	1	2	3	4
15	I had trouble falling asleep.	0	1	2	3	4
16	I had waves of strong feelings about it.	0	1	2	3	4
17	I tried to remove it from my memory.	0	1	2	3	4
18	I had trouble concentrating.	0	1	2	3	4
19	Reminders of it caused me to have physical reactions, such as sweating, trouble breathing, nausea, or a pounding heart.	0	1	2	3	4
20	I had dreams about it.	0	1	2	3	4
21	I felt watchful and on guard.	0	1	2	3	4
22	I tried not to talk about it.	0	1	2	3	4

Perceived Stress Scale

Cohen, S., Kamarck, T., Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, 24, 385-396.

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

	Never	Almost never	Some-times	Fairly often	Very often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Study ID # _____

~~A Pilot Study of Perceived Stress and Surgical Cytokine Patterns~~
Visual Analog Scale of Perceived Stress

How much do you feel stressed?

Not at all _____ Extremely

Valentina Sage Lucas
8345 Studley Road
Mechanicsville, VA 23116
Home: (804) 746-7123
VSLUCAS@vcu.edu

EDUCATION:

Virginia Commonwealth University/Medical College of Virginia, School of Nursing, Richmond, VA.
Currently PhD (candidate) in Nursing, expected graduation Fall 2012.

Virginia Commonwealth University/Medical College of Virginia, School of Nursing, Richmond, VA.
Dual Master of Science in Adult Health Nursing and Psych/Mental Health Nursing, May 1999.

Virginia Commonwealth University/Medical College of Virginia, School of Nursing, Richmond, VA.
Bachelor of Science in Nursing – *Summa Cum Laude*, December 1997.

Virginia Commonwealth University, School of Humanities and Sciences, Richmond, VA.
Bachelor of Science in Biology, December 1990.

PROFESSIONAL LICENSURE AND CERTIFICATIONS:

Registered Nurse, Virginia # 0001157454

Adult Health Nurse Practitioner, Virginia #0024164023

Authorization to Prescribe, Virginia #0017136880

CPR for the health professional

DEA#ML0704242

Certified Adult Nurse Practitioner, American Nurses Credentialing Center #342425-21

WORK EXPERIENCE:

Virginia Commonwealth University Health System, Division of Plastic Surgery

Adult Nurse Practitioner, March 2000 to present

- Provide care for the plastic surgical patient as well as individuals with chronic and difficult to heal wounds. Assess, treat and case manage.
- Provide education to residents, fellows, nurses and students in a variety of settings and formats.
- Provide home health visits for individuals with wounds who are physically unable to come to the clinic.
- Provide pre-op and post operative and follow up care to plastic surgery patients
- Current procedures approved to carry out include sharp debridement, biopsy, laser procedures, reconstructive nipple tattooing, and sclerotherapy
- Research coordinator for ongoing clinical trials for the practice

Virginia Commonwealth University, School of Nursing, Dept of Adult Health Nursing Systems

Graduate Adjunct Faculty, August 2010 to present

- Course faculty for NURS 676: Adult primary practicum
- Course faculty for NURS 679: Adult acute practicum

Hanover Medical Group, Mechanicsville, Virginia.

Adult Nurse Practitioner, June 1999 to March 2000

- Internal medicine/infectious disease practice addressing a wide variety of illnesses including, but not limited to, HTN, diabetes, HIV, hepatitis, STD's, asthma, COPD, bronchitis, allergies, dermatological concerns, obesity, sports medicine, well woman and routine physical exams, minor surgeries such as skin biopsy and suturing.
- Address a variety of mental health issues, such as depression, bipolar disorder, anxiety and substance detox.

Virginia Commonwealth University/Medical College of Virginia, School of Nursing, Richmond, VA.

Graduate Research Assistant for PNI Based Stress Management Research, Feb. 1998 - May 1999.

- Psychoneuroimmunology based research involving stress management in individuals with HIV.
- Primarily responsible for participant recruitment, scheduling and study promotion in the community.

Medical College of Virginia Hospitals, Richmond, Virginia.

Registered Nurse in the Woman's Specialties Unit, January 1998 to June 1999.

- Care of a variety of patient conditions, including but not limited to gyn-surgery, gyn-oncology, administer chemotherapeutic agents, care of general medical conditions and postpartum care.

Medical College of Virginia Hospitals, Richmond, Virginia.

Nursing Assistant in the Burn – Surgical Trauma Intensive Care Unit, Dec. 1996 – Dec. 1997.

- Assisted in care of patients in the burn and surgical trauma ICU, which included, but was not limited to, wound care, burn debridement, am care, feeding and ambulation.

McGuire Veterans Administration Medical Center, Lipid Research, Richmond, Virginia.

Biological Science Laboratory Technician, October 1991 – December 1996.

- Conduct research in cholesterol metabolism in in-vivo and in-vitro models, excelling in molecular applications.
- Represented lab at national meetings.

PUBLICATIONS

- Lucas, V.S. (2011). Psychological stress and wound healing in humans: What we know. *WOUNDS: A Compendium of Clinical Research and Practice*. 23 (4), 76-83.
- Lucas, V.S. & King, A. (2010) Wound healing for the plastic surgery nurse. *Plastic Surgical Nursing*, 30(3), 158-169.
- Pozez, A.L., Aboutanos, S.Z., **Lucas, V.S.** (2007) Diagnosis and treatment of uncommon wounds. *Clinics in Plastic Surgery*, October, 749-764.
- McCain, N.L., Munjas, B.A., Munro, C.L., Elswick, R.K., Robins, J.L., Ferreira-Gonzalez, A., Baliko, B., Kapowitz, L.G., Garrett, C.T., Brigle, K.E., Kendall, L.C., **Lucas, V.S.**, Cochran, K.L. (2003). Effects of stress management on PNI-based outcomes in persons with HIV disease. *Research in Nursing and Health*, March 102-117.
- Pandak, W.M., Stravitz, R.T., **Lucas, V.S.**, Heuman, D.M., Chiang, Y.L. (1996). HepG2 cells: A model for studies on regulation of human cholesterol 7-alpha-hydroxylase at the molecular level. *American Journal of Physiology*, March 401-410.

ABSTRACTS

- Aesthetic Inquiry: An emerging qualitative method. V. Lucas, J. DellaRipa, B. Biddle. School of Nursing, Virginia Commonwealth University, Richmond, VA 23298. Presented at Southern Nursing Research Society Meeting, Baltimore, MD, February 2009.
- Cytokine patterns, neuroendocrine mediators, and psychosocial variables in HIV disease. N. McCain, B. Munjas, C. Munro, R.K. Elswick, P. Kimball, A. Ferriera-Gonzalez, L. Kaplowitz, E. Fisher, C. Garrett, K. Brigle, L. Kendall, **V. Lucas** and J. Robbins. School of Nursing, Virginia Commonwealth University/Medical College of Virginia, Richmond, VA 23298.
- Alterations in cholesterol/bile acid homeostasis following over expression of cholesterol 7-alpha-hydroxylase using a CMV-C7alphaH adenoviral construct. C. Schwarz, D. Mallonee, Z.R. Vlahcevic, P.B. Hylemon, **V. Lucas**, J. Y. L. Chiang, K. Valerie and W.M. Pandak. Departments of Medicine and Microbiology, Virginia Commonwealth University/Medical College of Virginia, Richmond, VA 23298.
- Expression of fibroblast growth factor in the developing nervous system. Neuberger, T.J., **Sage, B.V.****, Russell, T., and DeVries, G.H. Dept. of Biochemistry and Molecular Biophysics, Medical College of Virginia. 23298
- Expression of fibroblast growth factor in the PNS during development and after injury. Neuberger, T.J., **Sage, B.V.**** and DeVries, G.H. Dept. of Biochemistry and Molecular Biophysics, Medical College of Virginia. 23298
- Fibroblast growth factor in the nervous system during development and after injury. Neuberger, T.J., **Sage, B.V.**** and DeVries, G.H. (Cornbrooks, C.J.) Dept. of Biochemistry and Molecular Biophysics, Medical College of Virginia. 23298

**Please note B.V. Sage is my maiden name.

CME PRESENTATIONS

- High Tech Whizardry and Wound Therapy: The Latest Technologies, Virginia Geriatrics Society Annual Conference, Williamsburg, VA 4/10/10
- Wound Healing for the Plastic Surgery Nurse, American Society of Plastic Surgical Nurses Annual Conference, Baltimore, 10/29/07
- Protecting the Skin: The Peri-operative Nurses Role, Association of Operating Room Nurses, Richmond Region, Richmond AORN Workshop, 2/24/07
- Wound Care Product Update, VCUHS Dermatology Grand Rounds, 11/06; 11/07; 2/08; 3/09
- Pressure Ulcers: Assessment and Treatment Strategies for the Spinal Cord Injured Patient, 15th Annual Spinal Cord Injury Symposium, Virginia Beach, VA 10/2/06
- Lower Extremity Ulcers, VCUHS Wound Healing Conference, Richmond, VA Fall 2005
- Wounds and Wound Care: Understanding Dressing Selection, VCU School of Medicine M4 update course, Annually Spring 2002 - 2005
- Uncommon Wounds, presented at VCUHS Wound Care Conference, Richmond, VA Fall 2003
- Options for Wound Care, VCUHS Dermatology Grand Rounds, annually from 2001-2005
- Nursing Considerations in Breast Reconstruction, Women's Health Clinical Byte, VCUHS Dept of Education and Professional Development. Fall 2004, Fall 2005 and repeated 10/26/06
- Wounds and Wound Care: A Primer, VCUHS Internal Medicine Grand Rounds, Fall 2004
- Wound Healing and Dressing Options, VCUHS Nurse Practitioner Grand Rounds, Spring 2002
- Options for Wound Care, VCUHS Geriatric Grand Rounds, Winter 2002

HONORS

- Editor's Award, Plastic Surgical Nursing, December 2011
- Southern Nursing Research Society's Dissertation Grant Award, February 2011
- VCU School of Nursing Doctoral Program Committee – student representative 2009-2011
- Barbara Farley Graduate Nursing Student Award – Spring 2009
- Hill-Rom Clinical Advisory Board, March 2009.
- VCUHS Excellence in Advanced Practice Nursing Award – Spring 2005
- Sigma Theta Tau Research Award – Spring 1999
- Professional Nurse Traineeship Funds – applied toward educational expenses for 1997 – 1999.
- VCU/MCV School of Nursing Promotion and Tenure Peer Review Committee – student representative 1999, 2007
- Graduated Summa Cum Laude, December 1997

COMMUNITY ACTIVITIES, ORGANIZATIONAL MEMBERSHIP

- Peer Reviewer – American Journal of Critical Care, Jan 2010
- VCUHS Tissue Use Committee (June 2009 – present)
- VCUHS Institutional Review Board (2006 – present)
- American Society of Plastic Surgery Nurses (2004 – present)
- Southern Nursing Research Society (2006 – present)
- Book Reviewer, Journal of Plastic Surgery Nursing (2006 – present)
- Planning Committee, VCUHS Biannual Wound Healing Symposiums (2003, 2005, 2007, 2009, 2011)
- Association for the Advancement of Wound Healing (2000 – present)
- Sigma Theta Tau Nursing Honor Society (1997 – present),
Nominations Committee Chair (1999-2001)
Vice-President (2001-2002)
Program Committee Chair (2001-2002)
- National and State Nursing Association (1998 – present)
- Co-Chair, VCUHS Standards of Practice Committee (2001-2002)

- Virginia Council of Nurse Practitioners (1997 – present)
Chair of Legislative Committee-Richmond Region (2001-2003)
- American Academy of Nurse Practitioners (1999 – present)
- Woodland Heights Civic Association (1992 – 2004)
- Fan Free Clinic – Volunteer health care provider (1999-2002)